Persistence of 2, 4-dichlorophenoxyacetic acid in some local soils

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M.Sc. in Ecology Dissertation

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ABSTRACT:

2,4-D is a commonly used herbicide applied over large areas of forest and agricultural land. Its persistence in the soil depends partly on the properties of that soil.

The persistence of 2,4-D in five soils, local to Durham and with differing soil properties, was studied. Although differences were found, problems were encountered with the method of assay of 2,4-D concentration, and it was thought that more time was required to use this method satisfactorily.

2,4-D sprayed on peat was much less available to seeds than it was when sprayed on the magnesium limestone soil, and was apparently either degraded very quickly, or locked up in the peat, with possibly a slow release later. However, it was possible to leach most of the 2,4-D out of the peat, as easily as out of the magnesium limestone.

2,4-D appeared to leach straight through a very dry soil, whereas much of it was held in the top soil when this was maintained at field capacity. Persistence of 2,4-D on the magnesium limestone in the field did not appear to be very different from that in the containers in the greenhouse.

Nitrogen content of the soil appeared to be unaffected by spraying with 2,4-D.

CHAPTER I INTRODUCTION

2.4 - dichlorophenoxyacetic acid (2,4-D) is a herbicide used widely in agriculture and forestry. It is now more commonly applied in combination with other herbicides than alone. This widespread usage raises ecological questions about toxicity, persistence and accumulation, and makes it particularly important to apply only the miniumum amount required for the desired effect. This amount will vary considerably in different situations and can only be accurately assessed in a given area by accurate knowledge of local edaphic and other environmental conditions, and of their influence on the persistence and effective toxicity of 2,4-D.

2,4-D remains active in the soil for varying amounts of time, ranging from two weeks to over eighteen months, depending on soil type and other environmental conditions (DeRose, 1946; Mitchell and Marth, 1946).

Loss of 2,4-D from the soil may be due to leaching, chemical breakdown or microbial degradation. Temporary loss of 2,4-D activity may also be due to adsorption onto soil colloids. Where leaching does not account for a large proportion of loss (Newman, Thomas and Walker, 1952), the pattern usually followed is:

(i) A rapid short-term loss of perhaps 10% of the activity in the first hour or two (Audus, 1949);

(ii) A variable lag phase in which the activity remains more or less constant;

(iii) Rapid degradation, at the end of which all significant activity is lost (Audus, 1951, 1952, DeRose and Newman, 1947; Newman, Thomas and Walker 1952; Torstensson, Stark and Goransson, 1975).

The initial phase is probably accounted for by adsorption onto soil colloids, and Audus (1952) found that up to .17m.g. of 2,4-D may be adsorbed for each gram of soil.

The lag phase suggests that the major degradation is due to microbial activity and there is much evidence for this (Audus, 1949 and 1950; Ogle and Warren, 1954; Helling, et al, 1968; Bollag et al, 1968; Loos et al, 1967a; Loos et al, 1967b) including work with sodium azide - a bacterial poison - and autoclaving (Audus, 1951; Brown and Mitchell, 1948; De Rose and Newman, 1947). The lag phase is considered to be the time required for bacterial cells to produce induced enzymes. It has been shown that soils previously treated with 2,4-D or related compounds (such as MCPA), or inoculated with bacterial cultures grown on 2,4-D, lose 2,4-D rapidly from the moment of application, without any lag (Audus, 1949, 1952; Torstensson et al, 1975; Newman and Thomas, 1949; Newman et al, 1952). Presumably this occurs because the microbial population has already built up the necessary enzymes. The period of lag has been shown to increase with soil depth (Newman et al, 1952). Where leaching is responsible for a large proportion of loss, there may be no lag if 2,4-D is applied in ester form; the initial breakdown to free acid, which may occur in as little as an hour and a half in the case of the isopropyl and n-butyl esters, is probably purely chemical (Smith, 1972).

Various bacteria capable of breaking down 2,4-D have been investigated. Studies of an Arthrobacter species, and of photodegradation of 2,4-D in aqueous solution, indicate that the most common initial Stage in breakdown is the loss of the alkanoic side chain to produce the phenol (Helling et al 1968; Loos et al, 1967a; Crosby et al, 1966, Loos et al, 1967b; Loos et al, 1967c), followed by dehalogenation to 4-chlorocatechol (Bollag, 1968; Crosby and Tutass, 1966) or 3,5 - dichlorocatechol (Tiedje et al, 1969). The studies of photodegradation of an aqueous solution suggested that 4-chlorocatechol was then further dehalogenated to 1, 2, 4 - benzenetriol, which was degraded without U-V light requirement, to polyquinoid humic acids (Crosby and Tutass, 1966).

Other workers (Tiedje et al, 1969,; Loos et al, 1967b) suggest breakdown by Arthrobacter of 4 - chlorocatechol and 3, 5 - dichlorocatechol to maleylacetic and chloromaleylacetic acid respectively. Other, but less common, pathways of degradation include some in which dehalogenation occurs without prior cleavage of the alkanoic chain (Crosby and Tutass, 1966). Some breakdown products are capable of stimulating plant growth. Flavobacterium aquatile is also capable of breaking down 2,4-D (Jensen and Paterson, 1952). Bacteria seem to retain their adaptation to degrade herbicides for considerable periods (Audus, 1952; Torstensson et al, 1975).

Generally, loss of 2,4-D is probably due largely to microbial degradation. Some workers suggest that leaching sometimes accounts for a large proportion of the loss where rainfall is heavy and drainage good (Ogle and Warren, 1954). Other research, however, suggests that even after heavy rainfall, little is lost from certain soils (Suffling et al, 1974), and it may be held very firmly onto soil colloids (Hanks, 1947; De Rose, 1946; Turner, 1971). Even after heavy leaching some activity may remain (Audus, 1951).

The time required for most of the 2,4-D to disappear certainly depends very much on soil type and condition. It is generally agreed that loss of 2,4-D increases as temperature rises, and as the soil's moisture content rises (Mitchell and Marth, 1946b, Ogle and Warren, 1954; Audus, 1949 and 1952; Brown and Mitchell, 1948; Kries, 1947; Jorgensen and Hamner,1948; De Rose and Newman, 1947; Crafts, 1949). Treated soil stored almost dry was found to be active after eighteen months (Mitchell and Marth, 1946b). The addition of manure up to about 4500Kg/ha was found to increase the rate of inactivation, but at about 9000Kg/ha the rate was markedly reduced (Brown and Mitchell, 1948). Light probably also affects persistence and effective concentration of 2,4-D, it being inactivated more quickly bright light (Penfound and Minyard, 1947; Crosby and Tutass, 1966).

Mixing 2,4-D with the soil is found to decrease its toxity in comparison with spraying it onto the soil.

Bioassays have been used extensively in the estimation of 2,4-D. The simplest method is the use of some measurement of growth of whole plants, from which a growth index is calculated; for instance, percentage germination (Brown and Mitchell, 1948); root growth expressed as a percentage fraction of the root growth of control plants over the same period. Maximum change in response usually occurs around the 50% growth response (GR_{50}) level (Hance and McKone in Audus, 1976). It is desirable to have a straight line relationship between concentration and growth response which can be achieved over a larger range if the logarithm of concentration is used. Species have been used in this way including Cucumber, (Cucumis sativus) (Newman and Thomas, 1949), cotton (Gossypium hirsutum) (Leonard at al, 1962), cress (Lepidium sativum) (Audus, 1952), sunflowers (Helianthus annuus) (Crafts, 1949), crabgrass (Digitaria sanguinalis) (Ogle and Warren, 1954), white mustard (Sinapis alba) (Torstensson et al, 1975), annual morning glory (I pomoea), barley (Hordeum vulgare) (Mitchell and Marth, 1946), and many other species.

2, 4 - D is a synthesized auxin. It causes differentiation and initiation of cell division in mature cells, in particular increasing division in the cambium and phloem, (Wiese and Rea, 1962), but it generally inhibits cell division in primary meristems of whole plants (Cartwright in Audus, 1976). This may partly be due to increased production of ethylene and partly due to the dislocation of vascular strands, leading to starvation of meristenatic tissue. Cell elongation occurs in shoots up to a certain concentration, after which it decreases. Growth is inhibited in the long term. Root growth is generally inhibited. Secondary meristematic activity is promoted in the vascular cylinder. Severe shrinkage in the protoplast in leaves of at least some species occurs, and chloroplast structure is destroyed (Bachelard and Ayling, 1971). It can also affect water soluble carbohydrate and nitrate content of leaves (Irvine et al, 1977). It is translocated in the phloem and plugging of phloem may eventually cause death.

Treated plants may show drooping, curling and distortion of leaves and stems (Jackson, 1962), abnormalities in leaf growth in some species, and epinasty. Sub-toxic amounts can cause increased growth, and may stimulate nucleic acid metabolism and protein synthesis.

Certain species are much more sensitive to 2,4-D than others. In general, monocolycotyledons are much less affected than dicotyledons, though a few, such as onion (Allium cepa) are very susceptible (Ogle and Warren, 1954). This may be due to the absence, in monocotyledons, of relatively undifferentiated tissue, such as vascular cambium and phloem parenchyma, which proliferate most easily. Broad-leaved species vary greatly in susceptibility and there does not seem to be any conclusive evidence as to which factors determine sensitivity. Attempts to equate it with quantity of cuticle wax (reported in Richardson, 1977) failed to produce any results, though stomata and ectodesmata are thought to allow for much uptake and their numbers may affect it.

It seems more likely that susceptibility is connected with uptake pattern, or ability to detoxify within the plant (Wiese and Rea, 1962), than with the amount taken up. When 2,4-D is applied to the leaves, some workers suggest that ability to restrict its subsequent movement to the shoot may partly confer resistance (Blackman, 1961; Leonard et al, 1962). However, others, studying four weed species differing in susceptibility did not find any correlation with translocation to shoot (Sanad et al, 1971). Although one fairly resistant species (Datura stramionum) did transport large amounts of 2,4-D after uptake through the leaf, Galium aparine, which is resistant, seemed to bind it immediately in the treated leaf. The two susceptible species studied (Chenopodium album and Galinsoga parviflora) showed intermediate patterns of translocation.

No correlation between translocation from roots to shoots and resistance seems to have been found. In general, little of the 2,4-D taken up by roots reached the shoots. In 32 hours, 3.8% of that absorbed by roots of Triticum vulgare was translocated to the shoots (Blackman, 1961).

However, there are marked differences found in uptake pattern. The most rapid uptake by roots of all species studied by Blackman occurred initially, often just for the first hour, though other workers have found longer initial periods up to five hours (Burns et al, 1969). After this, the rate of uptake was slower, becoming negative for many species. In resistant species (Triticum vulgare, Hordeum vulgare, Avena sativa, Oryza sativa, and the dicotyledon Linum usitatissum) the uptake increased again after this, though not to its initial level (Blackman et al, 1959; Shone, pers. comm.). With susceptible species the third stage of rate of uptake was negative, there being an egress of 2,4-D back into the external solution.

Uptake and translocation of 2,4-D appears to be at least partly metabolic (Shone and Wood, 1973). Shone and Wood found active uptake to be much greater at pH4 that at pH6.5, and associated this with the difference physico-chemical properties of 2,4-D at these two pH values, it being lipophilic at the lower pH and lipophobic at the higher. They suggested that lipophilic substances can diffuse into the vacuoles of cortical cells, whereas lipophobic compounds reach the shoots mostly from the free space in the roots.
Uptake and translocation is affected by environmental conditions. Relative humidity was found to increase absorption and translocation in Wolftail (Carex cherokeensis), the absorption possibly being increased due to increased permeability through the hydrated leaf cuticle (Burns et al, 1969). Increase in temperature, at least up to 20 or 30°C has been found to increase uptake, though above this it may be inhibited (MacKenzie et al, 1976; Burns et al, 1969; Hewitt and Curtis, 1948). Light intensity has also been found to affect toxicity in some species. Necrosis and epinasty of water hyacinth was found to be much greater in shade than full sunlight, though much less difference between reactions under varying light intensity was found in red kidney bean (Penfound and Minyard, 1947). Heather was also more susceptible with shade (MacKenzie et al, 1976). For some species, penetration into leaves was found to increase with light intensity (up to 20,000 lux), but for others there was no such simple relationship, though different intensities appeared to alter penetration (Richardson, 1977).

Translocation is reduced by water stress, though uptake is not affected. The chemical form of 2,4-D applied, and the addition of surfectants or other substances, also affects translocation and absorption.

In general, 2,4-D appears to be most potent when conditions are optional for growth (Mackenzie, 1976; Cartwright in Audus, 1976). Some species, resistant at a later stage, are susceptible as seedlings, including Triticum vulgare and other monocotyledons (Mitchell and Marth, 1946a). Translocation patterns also seem to vary at different times in a plant's growth.

2,4-D is degraded in plant tissues and disappears after a time (Loos et al, 1967a). It was found to disappear from cucumber in as little as 24 h. (Slife et al, 1962), but applied to Veronica baldwinii it remained from 4 - 12 weeks in the tissues (Rojas - Garciduenas et al, 1962). Many studies do not differentiate between 2,4-D and breakdown metabolites while these reamin in the plant. 2,4-D appears to remain in its active form in the plant for longer than IAA, which may partly account for its efficacy as a herbicide (Andraea and Good, 1957).

It was intended in the present study to look at persistence and movement of 2,4-D in the top 10cm of five local soils. Each soil was divided into three layers, the top 2cm, 2-6cm below the surface, and 6-10cm below the surface. The field capacity of each soil would be calculated and each of the soils analyzed at the three depths for pH, cation exchange capacity, and particle size. Any correlations between these properties and persistence or vertical movement of 2,4-D was to be assessed. Since watering regimes in the soils could not be fully standardized in this experiment, a comparison of different watering regimes would also be carried out. A brief comparison with one of the soils under field conditions would be made, in case leaching or some other environmental factor caused field persistence to be drastically different from greenhouse persistence. Any conclusions about 2,4-D applied on its own would probably be equally valid for its persistence when applied in herbicidal combinations (Smith, 1979).

The assessment of 2,4-D concentration would be conducted using bioassays. The uptake by seeds used in the bioassay on two of the five soils would be examined radio-chemically, to estimate whether the effect of 2,4-D on growth of the seed was accurately reflecting the concentration of 2,4-D put on the soil, or whether 2,4-D was being made unavailable to the seed before the bioassay was completed.

Leaching effects on the soil and seed would also be examined radio-chemically.

Finally, a comparison would be made of the effects of treatment of soil with 2,4-D on the nitrogen content of the soil.

Site Description:

The five sites were chosen to include some typical local soils, in particular the magnesium limestone, and for variation in soil type. These sites were: Thrislington Common; an upland peatmoor about two miles from the Derwent Reservoir; and three plots at the University of Durham Botanic Garden: an established turf; a garden loam; and a woodland soil.

It was ascertained that none of the plots from the Botanic Garden had been sprayed with any form of herbicide in the last year. Preliminary observations and tests suggested that the soils chosen varied in pH, drainage, humus and texture. None of the sites was on a noticeable gradient.

Site 1 - Thrislington Common:

Grid reference 337 338 (Ordnance Survey sheet 93). Approximate altitude: 150m.

This area is on magnesium limestone. The site chosen was in a well-frequented part of the common, where the local villagers walk their dogs and the children ride mopeds. It was not on the main pathways or cycle tracks, however, and by mid-summer the vegetation - largely Dactylis glomerata - was two or three feet high. Other species included Agrostis spp Festuca sp, Achillea millefolium, Ranunculus spp, Plantago lanceolata, Potentilla erecta.

Botanic Gardens:

Grid reference: 273402 (Ordnance Survey sheet 88).

Approximate altitude: 51m.

Site 2 - Turf:

This was part of a small area of established turf surrounded by cultivated plots. The soil was light coloured and clayey and the site poorly drained. Species included Agrostis spp, Festusca spp, Achillea millefolium, Plantago lanceolata, Bellis perennis, Trifolium spp.

Site 3 - Wood:

This was a shaded site at the edge of a small wood. The soil was friable and well-drained and covered with a layer of unhumified litter. Species included Urtica dioica, Veronica sp, Cirsium sp, Stachys sylvaticus, Senecio sp, Rumex sp, Chenopodium sp, Epilobium sp.

Site 4 - Loam:

This was part of a vegetable plot which had been dug and reseeded with grass a few months previously. The soil was friable and well-drained. Grass seedlings were just emerging when it was collected (late May) together with such weeds as: Tussilago farfara, Cerastium sp, Senecio sp, Chenopodium sp, Achillea millefolium.

Site 5 - Peat:

Grid reference: 962 471 (Ordnance Survey sheet 87). Approximate altitude: 425m.

This site was on a managed upland heather moor in an exposed position. The peat was sometimes only a few centimetres deep (5-10). It was underlain with a gravelly or sandy mineral soil and rock. The vegetation was almost exclusively Calluna vulgaris.

CHAPTER 2 MATERIALS AND METHODS

1. Preliminary Soil Tests:

Soils from five sites at the Botanic Gardens, one site on the Science Site and from an upland heather moor were collected. Initial tests were made for pH, organic carbon loss on ignition at low temperature (Ball, 1964), and oxidizable organic carbon. A few preliminary tests were also made for growth of wheat (*Triticum vulgare*) and cress (*Lepidium sativum*) on the soils when treated with 2,4-D.

2. Soil Collection:

An area of $100m^2$ was marked out at each site and samples taken from random points within that area.

Initial attempts were made to collect soil by means of a borer but it proved impossible to collect samples of sufficient depth from the more compacted soils by this means. Samples were taken by means of a trowel or spade to a depth of approximately ten centimetres and collected into polythene bags. The soil structure was preserved, if possible. Where this proved to be too difficult, owing to the soil's texture, the samples were separated on site into the three depths to be examined: 0-2cm, 2-6cm and 6-10cm below the surface, or put directly into the plastic containers. (See below).

3. Preparation of Soil for Analysis:

Soil from each of the five sites was separated into top, middle and bottom soil. Top soil was soil from the top two centimetres; middle soil from between two and six centimetres below the surface; bottom soil from between six and ten centimetres below the surface.

The fifteen soils were laid out in trays in the greenhouse to air dry. When thoroughly air dry (after at least a fortnight), samples were taken from each tray by repeated spreading and quartering, until a suitable quantity was obtained.

The samples thus obtained were sieved through a 2mm mesh and stored, where not immediately required, in covered plastic containers in the laboratory. Except where otherwise stated, these samples were used for analysis and for control bioassays.

4. Preparation of Soils for Spraying with 2,4-D:

Plastic containers, of the kind used for selling cream in, were obtained in bulk from Mono Containers. They were 11.5cm. deep, and of 6cm. diameter at the top, 5.5cm. diameter at the base. Between seventy and eighty plastic containers were filled to about 1cm. from the top with soil from Sites (2), (3), (4) and (5), and about a hundred and forty filled similarly with soil from Thrislington Common Site (1). Care was taken in filling the containers to preserve the original soil structure as far as possible and not to leave gaps at the sides of the container. Vegetation was left intact where possible.

Soils were watered once a week with tap water to approximately field capacity. The determination of field capacity is described in Section 7.2. When the greenhouse was exceptionally hot they were also watered more lightly in mid-week. The only exception to this watering regime were the containers used to try to compare the effect of soil water content on persistence of 2,4-D. (See below).

5. Spraying with 2,4-D:

5.1 Initial 2,4-D Persistence Experiment:

Fresh 2,4-D solution was made up for the spraying of all major bioassay experiments.

A solution of 100ppm 2,4-D was made up by dissolving 0.1g 2,4-D in 1.5cm^3 of 100% methanol and diluting to 1dm^3 with distilled water. 30cm^3 of this solution was added to thirty six containers of each of the soils from sites (2), (3), (4) and (5), and to forty containers of soil from site (1), on 25th June.

The 2,4-D solution was added directly to the soil surface by a $50cm^3$ syringe without a needle. Six containers partly filled with the 2,4-D solution but without soil were left in the greenhouse as controls.

5.2 Comparison of 2,4-D Persistence in Wet and Dry Soils:

Twenty-four more containers of soil from Thrislington Common (site (1)) were similarly sprayed with 100ppm 2,4-D solution on 9th July. Twelve of these containers had been watered to approximately field capacity before spraying with 2,4-D and were kept near field capacity by watering several times a week during the four weeks that samples were taken for testing. The remaining twelve containers had not been watered since 2nd July and were not watered again during the ensuing four weeks of the experiment.

5.3 Second 2,4-D Persistence Experiment:

A further twelve containers each of soils from sites (1), (3) and (5), (Thrislington Common, Wood and Peat soils) were sprayed with $30cm^3$ of 100ppm 2,4-D on 23rd July so that controls could be included with each weekly bioassay. (See 6.2.3).

5.4 Thrislington Common Field Experiment:

A $100m^2$ area of Thrislington Common, very close to the site of soil collection, was marked out. Within this area, ten random $100cm^2$ quadrats were marked with plastic pegs on 31st May. The $100cm^2$ quadrats were sprayed with 100ppm 2,4-D at the rate of $1cm^3$ solution to every cm^2, in a manner similar to that used with soil in the greenhouse. The process was repeated on 27th June with different $100cm^2$ quadrats within the same $100m^2$ area, and again on 27th July. On 27th July the quadrats were chosen to lie approximately on a diagonal of the $100m^2$ area, about 1m apart, owing to the difficulty of finding the random quadrats.

6. Bioassays:

6.1 Preliminary Experiments:

6.1.1 Different Species:

Twenty 9cm petri dishes were set up, with a 9cm Wheatman's number 1 filter paper in each. Seeds of radish (Raphanus sativus)variety 'French Breakfast'; lettuce (Latuca sativa)variety 'All the Year Round'; cress (Lepidium sativum)variety 'Extra curled'; wheat (Triticum vulgare), and pea (Pisum sativum) were put to germinate on the filter papers. Five dishes were watered with distilled water as controls; five with 1ppm 2,4-D solution; five with 10ppm 2,4-D solution; and five with 100ppm 2,4-D solution. Each of the five dishes at the same concentration contained seeds of a different species. All dishes were watered with $4cm^3$ of solution except those containing the peas which were watered with $5cm^3$. After 3 days, $3-4cm^3$ distilled water was added to each dish except those containing the peas, to which $4-5cm^3$ were added. The peas were observed over a six day period to estimate which species was likely to be the best indicator of 2,4-D concentration. Sections of radical and epicotyl were also examined under the microscope to observe the effect of 2,4-D on cell expansion and division.

These initial observations indicated that wheat and cress would show the best variation in that range of 2,4-D concentration. Fresh petri dishes and filter papers were prepared. Two dishes, one containing wheat and one cress seeds, were set up at each of the following concentrations of 2,4-D: 0ppm (pure water), 1ppm, 2.5ppm, 5.0ppm, 7.5ppm, 10ppm, 25ppm, 50ppm, 75ppm and 100ppm. The length of shoot and root was measured for both cress and wheat to find which gave the best range of values.

Oat (Avena sativa) seeds were obtained a few weeks later, but germinated too slowly to be of use.

6.1.2 Best Measurement for . . . Index:

Having ascertained that wheat seemed to give the best easily measured variation over the range of concentrations required, graphs were plotted of the length of shoot, length of radicle, and combined length of shoot and radicle against the logarithm of the concentration of 2,4-D, to estimate which measurement gave the best range of values and was the nearest to a straight line fit.

6.1.3 Comparison of Germinated with Ungerminated Seeds for Bioassay:

From the measurements described in 6.1.2 it was decided the shoot was the best assessment of concentration, having a higher coefficient of correlation than the root and being easier to measure than shoot + root, which had the same coefficient.

Owing to the large standard deviations obtained when estimating the mean of shoot growth of previously ungerminated seeds at a particular concentration of 2,4-D, seeds were germinated for two days prior to being placed in petri dishes containing soil from each of the five sites at each of the three depths at different concentrations of 2,4-D. The growth of these seeds over two days was measured and the variation compared with that of the growth of previously ungerminated seeds.

6.1.4 Number of Days for Growth:

As previously germinated seeds did not show significantly less variation, ungerminated seeds were used in the bioassays.

Previously ungerminated seeds were measured after two, three and four days growth in each of the fifteen soil types at different 2,4-D concentrations.

Graphs were drawn of shoot length after three and four days growth against the logarithm of 2,4-D concentration for each soil type, to ascertain whether the extra days growth gave significantly clearer results.

6.1.5. Position of Petri Dishes:

Attempts were made to set up petri dishes in a near vertical position. This method was suggested by Parker (1964) (quoted in Audus, 1976), in order to encourage the seedlings to grow against the cover of the dish, thus facilitating measurement.

6.1.6 Soil Controls:

A thin layer of soil from each of the fifteen types was put on filter paper in four 9cm petri dishes, and one dish of each soil type watered in 15cm^3 of: distilled water, a 10 ppm solution of 2,4-D, a 50 ppm solution of 2,4-D and a 100 ppm solution of 2,4-D. The shoot index at each concentration for each soil type was calculated by:

$$\text{Shoot index for soil A at concentration x ppm of 2,4-D} = \frac{\text{mean length of shoot of seeds in soil A at concentration x}}{\text{mean length of shoot of seeds in soil A at zero concentration}}$$

Graphs were plotted of shoot index against logarithm of concentration, and the best straight line fit obtained. The correlation coefficient, and significance of fit using the Student's t test were also calculated.

6.1.7 Cucumber Seeds:

Cucumber seeds (Cucumis sativa) have been used extensively in bioassays for 2,4-D (Ready and Grant 1947; Newman, Thomas and Walker, 1952), and are very sensitive to low concentrations of the herbicide (Slife et al, 1962). Similar preliminary tests, as already described for wheat, were carried out with cucumber seeds measuring the radicle: comparison of germinated with ungerminated seeds; comparison of growth over different numbers of days; controls, initially just on filter papers and later on soils; measuring the radicle at different concentrations of 2,4-D. It was hoped thereby to assess 2,4-D concentrations below 10ppm, below which wheat seeds showed little, if any, inhibition in growth.

However, owing to problems with supply (the initial suppliers recalled their seeds) and the cost of large quantities of seeds, cucumber seeds were eventually used only occasionally as an additional possible check for traces of 2,4-D to which the wheat seeds might no longer be responding. In analysing the results, it was decided that these measurements were too variable to warrant consideration as it had not been possible to use enough seeds, and they were discarded.

6.2 Weekly Bioassays:

6.2.1 Initial Bioassays:

Weekly bioassays were made of the concentration of 2,4-D remaining in the soils in the containers, the first bioassay being started a few hours after the soils were sprayed on 25th June. It was thus hoped to obtain an estimate of the equivalent concentration of 2,4-D remaining active in the soils at weekly intervals, and to thereby compare the rate of degradation of 2,4-D in each soil and the number of weeks required for it to become inactive. It was also hoped to look at vertical movement of 2,4-D in the soil. Three containers were taken at random from the containers of each soil type. The soil in each container was divided into top, middle and bottom soil (as described previously). Soil from each of these three divisions was put onto a filter paper in a 9cm petri dish, so as to form a thin layer. Large pieces of vegetation and stones were removed, and the soil crumbled if lumpy. Thus there were three replicate petri dishes for each of the fifteen soil types. Five wheat and, where used, three cucumber seeds were placed on the soils (the cucumber seeds embedded sideways in the soil) in each dish. Each dish was watered with sufficient distilled water to bring it to about field capacity, where it was not already so. The dishes were left covered for three days and placed horizontally in the laboratory where it was thought conditions would be less variable than in the greenhouse. Occasionally a dish was rewatered during the three days where it had dried out significantly, but normally this was not necessary.

After three days seeds were removed and the length of shoot measured and recorded. The mean and standard deviation of shoot length for each soil type was assessed. Shoot indices were also assessed using the original controls, and the means and standard deviations calculated. This was continued for seven weeks, eight bioassays being made in all. For three weeks (starting on 25th June), until it evaporated, bioassays were also made on the solution left in six containers in the greenhouse. (See 5.1).

6.2.2 Bioassays Comparing Wet and Dry Soils:

Similar weekly bioassays were performed for four weeks starting on July 9th on the twenty four containers of soil from site (1) sprayed on 9th July. It was hoped to thus compare persistence and rate of breakdown of 2,4-D in wet and dry soils. It was thought that differences in watering might partly explain the variation between bioassays from different containers since, owing to the size of the initial experiment, it was impossible to accurately measure the water given to each container. The two watering regimes in this experiment thus presented extremes: very different, not only from each other, but also from the watering of the initial experiment.

6.2.3 Second Bioassays:

Owing to the difficulties of setting up so many dishes and taking so many measurements at once, weekly control bioassays in uncontaminated soil were not set up on 25th June. However, weekly variation in bioassay results in the first four weeks was so great that it was decided controls were necessary to try to check whether variations were caused by changes in 2.4-D taken up by the seeds or by changes in laboratory conditions.

On 23rd July, and for the ensuing four weeks, controls were included and bioassays were also made of the nine soil types sprayed on 23rd July. (See 5.3 above). Thus, 23rd July was the start of the first bioassay (week 0) on the newly sprayed soil from sites (1), (3) and (5), and of the fifth bioassay of the soils sprayed on 25th June. The same controls could be used for each to estimate shoot indices for week 0 and week 5.

6.2.4 Thrislington Common Field Experiment:

Weekly bioassays on sprayed quadrats and on controls (unsprayed samples from within the 100m^2 site) from Thrislington Common (see 5.4) were made for four weeks, or until 2,4-D had virtually disappeared, after the two initial sprayings (31st May and 27th June), and for two weeks after the final spraying (27th July). However, the assays made in June were not consistent with the other experiments. As the method of bioassay had not been finalised it is only possible to compare sprayed with unsprayed samples. Soil for the bioassay was taken from the top four centimetres.

This experiment was set up merely to look for drastic differences in persistence of 2,4-D between greenhouse (undrained) conditions and field conditions. There was no attempt to obtain accurate measurements of concentration, nor to compare different depths.

7. Soil Analyses: (See Appendix for details of reagents).

7.1 pH:

The pH of each soil type was measured using the pH metre in the Geography Soil Laboratory. Two measurements were made for each soil type (Hesse, 1971; Soil Laboratory Handbook): 20g of soil was made into a paste with KCl, and 10g of soil was stirred with $10cm^3$ distilled water; both paste and suspension were allowed to stand for an hour before measuring pH.

7.2 Field Capacity:

Field capacity was measured for soil from each site taken as a whole and not separated into top, middle and bottom. Glass beakers were weighed empty. They were then partly filled with soil. Water was slowly added to the soil in each pot, stirring continuously with a glass rod, until field capacity was thought to have been reached. After weighing, the beakers were placed i the oven at $105^{\circ}C$ for 26 hrs to allow the soil to dry thoroughly, then reweighed. At least two results were obtained for each soil to obtain a mean, and giving a coefficient of variance of less than 4%.

7.3 Organic Carbon: (Hesse, 1971, Fundamentals of Soil Science, Laboratory Handbook).

7.3.1 Walkley-Black Wet Oxidation:

This was measured by the Walkley-Black method of wet oxidation for soils from sites (1), (2), (3) and (4). About .5g of .15mm soil was weighed and put into a 500ml conical flask. $10cm^3$ of .17 potassium dichromate was added as oxidising agent and the flask swirled to mix thoroughly. $20cm^3$ of concentrated sulphuric acid was added quickly in a fume cupboard and the mixture again swirled. After leaving the flask to stand for thirty minutes, $200cm^3$ of distilled water and $10cm^3$ of concentrated 'O' phosphoric acid were added.

When the mixture had cooled 15 drops of barium diphenylamine sulphonate indicator were added and the solution titrated against .5M ammonium ferrous sulphate until the colour changed from dark blue to clear green. The method was repeated without soil to standardise the ammonium ferrous sulphate.

7.3.2 Loss-on-ignition:

Organic carbon of the peat soils was measured by loss-on-ignition and Ball's regression (Ball, 1964).

Air-dry soil was left at 110°C for two days to drive off water. A quantity was then immediately weighed, put into a silica dish, and placed in a muffle furnace at 375°C for 16 hrs. It was removed from the furnace, placed in a dessicator to cool, then reweighed. The percentage loss-on-ignition, by weight, was calculated and the organic carbon content obtained using Ball's regression.

7.4 Particle Analysis: (Methods of Testing Soils for Civil Engineering Purposes, 1967).

This was done by the pipette method.

A quantity of the soil to be analysed (between 12 and 30g) was accurately weighed to .001g. The soil was put in a 500cm^3 conical flask and 50cm^3 distilled water added. It was boiled to approximately 40cm^3. When cool, 75cm^3 of hydrogen peroxide (20 volume solution) was added to destroy organic matter. The mixture was covered and left overnight. It was then gently heated, care being taken to avoid frothing over, and frequently swirled. When most of the frothing had subsided, it was reduced to 30cm^3 by boiling. The mixture was filtered using a Buchner funnel. The soil was transferred, using jets of distilled water, from the filter paper and sides of the funnel to a weighed evaporating dish. It was dried thoroughly at 105-110°C and reweighed.

The soil was dispersed using 25ml of sodium hexametaphosphate solution and 25ml of distilled water. The mixture was stirred with a rubber policeman, then put back in the oven to warm gently for ten minutes. It was transferred with a jet of distilled water into a dispersion cup and mixed mechanically for ten minutes. The suspension was filtered through a number 200 (75 micron) BS test sieve, and washed through with distilled water, care being taken not to use more than 150cm^3 of water. The suspension was transferred to a graduated sedimentation tube and made up to 500cm^3 with distilled water. The material retained on the sieve was dried at 105-110°C then resieved using sieves numbers 25 (600 microns), 72 (210 microns) and 200 (75 microns). The material retained on each sieve was weighed.

The sedimentation tubes containing the soils were put in a constant temperature water bath and rubber bungs inserted. They were left to acquire the temperature of the bath. They were then shaken thoroughly, a stop-watch being started at the same time as the first tube was shaken.

Three pipettings were taken from each tube; the first at 4m5s, the second at 46m, and the third at 6h45m after starting the stop-watch. The pipettings were made by clamping the pipette so that it was just above the surface of the suspension, then very gently lowering it until it was the requisite depth below the surface, and reclamping. The suspension was drawn into the pipette by opening the appropriate tap, which was then closed, and the pipette gently removed. Thus the suspension was disturbed as little as possible. The contents of the pipette were put in a weighing bottle and any suspension left on the walls of the pipette washed down with a small amount of distilled water.

The weighing bottle and contents were dried at 105°-110°C then weighed to the nearest .001g so that the weight of solid material for each pipetting of each sample could be determined.

The weight of solid material in a pipetted sample from a tube containing 25cm^3 sodium hexametaphosphate, and made up to 500cm^3 with distilled water, had been pre-determined.

7.5 Cation Exchange Capacity:

Bascombs' method was used as this is suitable for all soil types.

1g of soil was weighed and put into a 50cm^3 centrifuge tube, sealed and weighed. The soils from Thrislington Common, being calcareous, were pretreated by shaking with buffered barium chloride for an hour, centrifuging for about quarter of an hour and discarding the supernatant. 40cm^3 buffered barium chloride was then added to all the soils and they were left overnight, centrifuged, and the supernatant discarded. 40cm^3 distilled water was added, the tubes shaken thoroughly, centrifuged, and the liquid again discarded. The tube, soil and seal were again weighed, 20cm^3 magnesium sulphate solution was pipetted into the tube, shaken for two hours, centrifuged, and the liquid transferred to a flask and sealed.

6 drops of 2M ammonium hydroxide were added to 5cm^3 of this liquid, 2 drops of catechol violet indicator added, and this titrated with EDTA solution until the colour changed from blue to reddish violet. 5cm^3 of the magnesium sulphate solution was similarly titrated for a blank determination.

7.6 Total Nitrogen: (See section 8) (Laboratory handbook).

This method is a modification of Kjeldahl's method (1883).

5g of soil were weighed and placed in a Kjeldahl flask. 10 potassium sulphate-selenium catalyst tablets were added, to increase the temperature of the reaction and therefore the speed of oxidation, followed by 25cm^3 of distilled water. The mixture was swirled and 30cm^3 concentrated sulphuric acid was added as oxidising agent. The flask was heated gently on a digestion rack until it changed to a greenish colour. This took about two hours. The flask was cooled and the contents washed into a 250cm^3 conical flask with distilled water and diluted to 250cm^3 with distilled water.

A Markham apparatus was used to distil 10cm^3 of this solution and 10cm^3 of 40% sodium hydroxide into 10cm^3 boric acid and mixed indicator, which turned from red to blue. When about 30cm^3 of distillate had been collected, it was titrated against .02M HCl until the colour changed to red.

7.7 Total Exchangeable Bases: (Hesse '71, Laboratory Handbook).

Metson's method was used.

Soil was extracted by leaching with neutral 1M ammonium acetate solution. A plug of absorbent cotton wool was put at the bottom of a leaching tube, the soil placed on top of it and compacted and another plug of cotton wool positioned on top of the soil. A volumetric flask containing the ammonium acetate was inverted over the tube with its neck inserted into the tube. The leachate was collected in a conical flask. 5g of soil were leached with 250cm^3 ammonium acetate, and the leachate made up to 250cm^3 with ammonium acetate solution. A blank, without soil, was also included.

A 25cm^3 aliquot of each leachate was evaporated to near dryness in a glass beaker, then transferred to a small silica basin, using a jet of distilled water, and evaporated to dryness carefully on an electric hotplate. When completely dry, it was heated in a muffle furnace at 500°C for an hour. 5cm^3 of .2M hydrochloric acid was added, and the residue suspended by stirring with a glass rod. It was digested on a water-bath for thirty minutes then titrated against .1M ammonium hydroxide solution. 5 drops of methyl orange indicator were used.

7.8 Exchangeable Calcium and Magnesium:

The leachate collected as described in section 7.7 was used to obtain one set of values (Laboratory handbook), and the final titrated solution from 7.7 for another (Hesse,'71) for comparison. 4cm^3 of the solution to be used were taken and 6 drops of lanthium chloride added. Calcium and magnesium were measured using the PERKIN-ELMER 403 absorption spectrophotometer.

8. Effect of 2,4-D on Nitrogen Content of Soil:

Using the method described in 7.6, soil from the top, middle and bottom sections of Thrislington Common was analysed for nitrogen content: unsprayed, three days after spraying, and ten days after spraying with 2,4-D. Soil to be analysed was dried at about 105°C overnight, sieved through a 1mm mesh (approx) sieve, and 5g were weighed out for analysis.

9. Radiochemical Analysis:

A solution of 100ppm unlabelled 2,4-D was made up as usual. .5cm^3 of 2,4-D ^{14}C, labelled in the chain, was added. The radioactive 2,4-D added had a specific activity of 50 μ Ci/CM^3.

A thin layer of top soil from Thrislington Common was put on two 20cm glass petri dishes, and a thin layer of top soil from the peat put on two others. Enough of the diluted radioactive 2,4-D solution was added and mixed with the soil to moisten the soil to field capacity (this involved adding 22cm^3 of the solution to the soil from Thrislington, and 15cm^{3}to the peat). Fifty live, but ungerminated wheat seeds were placed on one dish of each soil, and fifty wheat seeds previously killed by keeping at 90°C for 24hrs were placed on each of the remaining dishes .

Two seeds were removed from each dish at intervals over the next three days. On removal they were washed in distilled water, dried, put in vials and the vials immediately put at -20°C and kept there until crushed and mixed with scintillation fluid. The experiment was started at 12.00 hrs on the 13th August. Seeds were removed at: 1230, 1300, 1330, 1400, 1430, 1500, 1530, 1600, 1800, 2000, 2200, 2400hrs. on the 13th August; at 0400, 1000, 1600, 2400hrs. on the 14th August; at 1000 and 2200 on the 15th August; at 1000 on the 16th August.

One or two days after removal from the radioactive soil, the seeds were taken from cold storage and the two seeds from each vial crushed with .4 - 1.0cm^3 distilled water and 10cm^3 scintillation fluid added.

Control samples of each soil type were also taken and $10cm^3$ scintillation fluid added to each.

Additional samples of each soil type were taken and shaken for approximately two minutes with distilled water and centrifuged. $1cm^3$ of the wash from each tube was put in a scintillation vial and $10cm^3$ of scintillation fluid added. The soil left after the supernatant liquid had been removed was washed into a scintillation vial using approximately 1ml of distilled water and 10ml of scintillation fluid. It was thus hoped to compare 2,4-D leached out by the water, and 2,4-D retained in the soil.

Two germinated seeds (shoot approximately .5cm) were taken from the Thrislington Common soil, washed, dried, crushed with $.5cm^3$ water and $10cm^3$ scintillation fluid added. Two similarly germinated seeds were shaken for ten minutes with $10cm^3$ distilled water, then treated as above, and another two shaken for thirty minutes with $10cm^3$ distilled water before being prepared for scintillation counting. It was thus hoped to look at the amount of 2,4-D removed by washing.

Counts per minute were made on a Beckman LS-200B scintillation counter. The contents of each vial were counted for five minutes, and every vial was counted twice to check there were no large discrepancies in readings.

10. Analysis of Results:

Where appropriate, the calculation of means and standard deviations of bioassay data, shoot indices and 2,4-D concentrations from bioassays, and the plotting of graphs for bioassay controls and data and for the radiochemical experiments were done using the NUMAC computer system. This involved use of SPSS,GHOST routines, the DURH: CURVEFIT routine and programming in Fortran.

Symbols used in Results and Appendices:

In all graphs, except those of uptake of radioactive 2,4-D by wheat seeds, different symbols for points on the graph represent soils from different depths.

△ represents soil from the bottom depth.

▽ represents soil from the middle depth.

□ represents soil from the top 2cm.

In graphs of uptake of radioactive 2,4-D:

△ represents seeds on soil from Thrislington Common.

▽ represents seeds on peat soil.

CHAPTER 3: RESULTS

1. Soil properties:

pH Values: (table 1)

There is a wide range of pH values between the soils from different sites, but not between soils from the same site at different depths. The peat, as expected, has a much lower pH than the other four. Looking at the results with the soils suspended in water, the turf, wood and loam soils are on the acid side of neutral, and Thrislington Common is slightly alkali. Some authorities recommend taking pH values of the soil made into a paste with potassium chloride, to standardize salt effects (Hesse, 1971). The results obtained by this method are included for comparison.

TABLE 1:

pH Values Results

Site	Soil Depth	pH of 10g of soil in $10cm^3$ of water	pH of paste of 20g soil in potassium chloride
Thrislington Common	Bottom	7.7	6.7
	Middle	7.5	6.6
	Top	7.6	6.7
Turf	Bottom	6.0	5.1
	Middle	6.0	5.0
	Top	5.8	5.1
Wood	Bottom	6.3	5.2
	Middle	6.1	5.0
	Top	6.2	5.4
Loam	Bottom	6.4	5.5
	Middle	6.6	5.9
	Top	6.8	6.0
Peat	Bottom	3.6	2.4
	Middle	3.4	2.4
	Top	3.5	2.5

TABLE 2:

Field Capacity Results

Soil type	% of water held at field capacity		Coefficient of variance %
	mean	standard deviation	
Thrislington Common	69.3	.14	.2
Turf	70.7	.14	.2
Wood	58.3	1.06	1.8
Loam	48.8	1.56	3.2
Peat	254.8	.42	.2

Organic Carbon: (TABLE 3)

There is an increase in organic carbon from bottom soil through to top soil, as would be expected. The top soil from Thrislington Common and the turf may have given slightly higher results than they should due to pieces of grass which were not separated from the soil by 2mm sieving. The results for peat may not be directly comparable to the other soils, as they were calculated by a different method. There was generally more variation between bottom and top soils from one site than between soils of different types.

Ball's regression (1964) for loss-on-ignition was based on studies of over a hundred soils of differing organic content, and although it may not be suitable for application to very different soils (Hesse, 1971), the soils studied here should be sufficiently similar.

TABLE 3:

Organic Carbon Results:

Results for Thrislington Common, Turf, Wood and Loam calculated by Walkley-Black method. Results for Peat calculated from loss-on-ignition.

	A	B
Soil Type	% oxidizable carbon (uncorrected)	Total organic carbon % B=Ax1.33
Thrislington Common Bottom	2.94	3.91
Thrislington Common Middle	3.25	4.32
Thrislington Common Top	4.40	5.85
Turf - Bottom	2.37	3.15
Turf - Middle	3.64	4.84
Turf - Top	4.30	5.72
Wood - Bottom	2.50	3.33
Wood - Middle	2.60	3.46
Wood - Top	3.50	4.66
Loam - Bottom	2.35	3.13
Loam - Middle	2.31	3.07
Loam - Top	2.70	3.59
Peat - Bottom		7.60
Peat - Middle		24.63
Peat - Top		37.87

It was not possible to obtain any results for the peat soils by the Walkley-Black method as the titration could not be seen to reach an end point.

TABLE 4:

Soil Particle Analysis: (Results corrected to one decimal place).

	SAND FRACTIONS			SILT FRACTIONS		
Soil Type	% on 25 sieve 2.4-0.6mm	% on 72 0.6-0.21mm	% on 200 0.21-0.075mm	Coarse 0.06-0.02mm	Medium 0.02-0.006mm	Fine 0.006-0.002mm
Thrislington Common:Bottom	15.8	26.5	15.7	34.3	5.3	0
Thrislington Common:Middle	5.8	27.1	19.8	37.5	3.9	2.7
Thrislington Common:Top	-------	No results	----------	----------	----------	----------
Turf:Bottom	3.4	9.1	16.9	43.2	6.3	1.6
Turf: Middle	5.6	10.4	17.3	42.7	3.4	3.0
Turf: Top	1.9	33.4	.3	42.1	5.6	2.6
Wood:Bottom	3.9	8.5	17.3	33.5	12.5	7.1
Wood:Middle	4.1	7.5	17.4	46.4	6.7	2.7
Wood:Top	2.3	26.4	0.0	42.0	7.2	5.2
Loam:Bottom	6.2	13.2	23.2	35.9	7.0	3.4
Loam:Middle	6.6	20.4	31.6	27.5	2.9	4.8
Loam:Top	5.4	18.7	33.7	27.7	2.9	4.7

Soil Type	CLAY less than 0.002mm	SAND total percentage	SILT total percentage
Thrislington Common:Bottom	2.5	58	39.5
Thrislington Common:Middle	3.3	52.7	44.0
Thrislington Common:Top	---------	No results	----------
Turf:Bottom	19.6	29.4	51.0
Turf:Middle	17.5	33.3	49.2
Turf:Top	14.2	35.6	50.2
Wood:Bottom	17.3	29.7	53.0
Wood:Middle	15.2	29.0	55.8
Wood:Top	16.9	28.7	54.4
Loam:Bottom	11.1	42.6	46.3
Loam:Middle	6.1	58.6	35.3
Loam:Top	6.8	57.4	35.8

Soil Particle Analysis: (Table 4)

The results for coarse silt are unrealistically high. They take into account any soil lost in the analysis, since they are calculated by subtraction of the other results from 100%. The organic matter was not completely destroyed in some of the soils by the pretreatment, and the fine particles were not always completely dispersed, so this might account for some of the error. However, it is felt that in spite of these inaccuracies, the results did give a good estimation of the differences between the soils.

Thrislington Common and loam soils showed similar proportions of sand, silt and clay, as did the wood and turf soils.

The peat soil was not analyzed for particle size, as it was thought to be too high in organic matter for the results to be meaningful.

TABLE 5:

Cation Exchange Capacity Results

Soil Type	Cation Exchange Capacity (C.E.C. in m.e./100g soil
Thrislington Common bottom	40.56
Thrislington Common middle	30.72
Thrislington Common top	34.4
Turf bottom	29.36
Turf middle	26.08
Turf top	30.8
Wood bottom	24.96
Wood middle	25.36
Wood top	27.28
Loam bottom	23.6
Loam middle	24.48
Loam top	No results
Peat bottom	37.84
Peat middle	19.44
Peat top	34.32

There is an unexpectedly wide variation between the bottom and middle soils of Thrislington Common, and the middle and peat soil and the peat from the top and bottom, and it seems likely that the middle peat result, at least, is invalid. There was difficulty in centrifuging the top and middle soils from the peat, as these were very light, and it was impossible not to throw a small amount out with the wash. This might partly account for the strange result. The high values for the top and bottom peat soils are presumably accounted for largely by exchangeable hydrogen, whereas Thrislington Common is high in minerals (see results for exchangeable calcium and magnesium). There is no marked pattern from bottom through to top, except for the wood, and possibly the loam, soils.

TABLE 6:

Total Exchangeable Bases

Soil Type	Total Exchangeable Bases in me/100g soil
Thrislington Common Bottom	44
Thrislington Common Middle	32
Thrislington Common Top	28
Turf Bottom	2
Turf Middle	0
Turf Top	44
Wood Bottom	20
Wood Middle	30
Wood Top	22
Loam Bottom	2
Loam Middle	2
Loam Top	4
Peat Bottom	20
Peat Middle	0
Peat Top	2

Total Exchangeable Bases: (Table 6)

The results obtained are very erratic, and possible explanations are discussed in Chapter 4.

TABLE 7:

Exchangeable Calcium and Magnesium Results

Results from leachate (see methods)

Soil Type	Calcium ppm	Calcium m.e./100g	Magnesium ppm	Magnesium m.e./100g
Thrislington Common Bottom	82	10.25	21.2	4.5
Thrislington Common Middle	85	10.63	16.9	3.5
Thrislington Common Top	96	12.00	18.9	4.0
Turf Bottom	54	6.75	5.6	1.2
Turf Middle	57	7.13	5.8	1.2
Turf Top	64	8.00	7.3	1.5
Wood Bottom	46	5.75	4.5	.9
Wood Middle	47	5.88	4.9	1.0
Wood Top	55	6.88	6.2	1.3
Loam Bottom	68	8.50	2.7	.6
Loam Middle	62	7.75	2.5	.5
Loam Top	63	7.88	2.8	.6
Peat Bottom	5	.63	0.7	.1
Peat Middle	9	1.13	1.9	.4
Peat Top	27	3.38	5.5	1.2

Exchangeable Calcium and Magnesium: (Table 7)

Exchangeable calcium generally increases from the bottom soil through to the top. Thrislington Common has the highest values, then the loam soil, turf, wood with the peat by far the lowest. This follows the order of pH values (from high to low) except that the positions of the turf and wood are reversed.

Magnesium is also higher generally in the top soil, except for Thrislington Common where the bottom soil has the highest values. As would be expected, Thrislington Common, on the magnesium limestone, has much higher concentrations of magnesium than any of the other soils.

TABLE 8:

Nitrogen Determinations Results

Soil Treatment	Soil depth	% nitrogen in sample (mean)	standard deviation	coefficient of variance %
Control	Bottom	.31	.069	22
	Middle	.36	.069	19
	Top	.44	.053	12
Sprayed with 2,4-D less than 1 week before nitrogen analysis	Bottom	.31	.020	6
	Middle	.38	.020	5
	Top	.43	.069	16
Sprayed with 2,4-D more than one week before analysis	Bottom	.31	.045	14
	Middle	.40	.025	6
	Top	.43	.000	0

Nitrogen: (Table 8)

This table shows any differences found between nitrogen content of controls (based on three samples); nitrogen content of soil three days after spraying with 100p.p.m. 2,4-D (based on two samples); and nitrogen content ten or eleven days after spraying (average of two samples).

TABLE 9:

2. Preliminary experiments for Bioassays

Comparison of suitability of measurements of shoot, root and shoot + root as indicators of 2,4-D concentration.

Best straight line fits of plotting logarithm of 2,4-D concentration from 1ppm to 100ppm along x-axis, and length in cm. of relevant measurement along y-axis.

Measurement taken for y-axis	Slope	y-intercept	Correlation coefficient
Shoot length in cm.	-1.1	3.1	-.93
Root length in cm.	-.37	.95	-.89
Shoot + root length in cm.	-1.5	4.1	-.93

Examination of sections of pea shoot and root under the microscope after one weeks growth in a solution of 100p.p.m. 2,4-D showed expansion of the stele and pith.

Plates 1 - 4 show the effect of 2,4-D on wheat roots after ungerminated wheat seeds had been grown for three days in a 100p.p.m. solution of 2,4-D. Although the stele appears to have remained intact, the cells of the cortex are very broken and structure has been lost.

2. Preliminary Experiments for Bioassays:

Lettuce, radish, pea, cress and wheat seeds were examined for growth at different concentrations of 2,4-D up to 100p.p.m. Only cress and wheat showed a suitable pattern of decrease in growth with increase in concentration. In a more detailed comparison of growth of cress and wheat seeds at different concentrations, the wheat proved a more suitable assay, since the cress was too sensitive to the herbicide at these concentrations, and its growth at concentrations above 2.5p.p.m. was so slight that differences could not easily be measured.

The correlation coefficients shown in Table 9 show that the measurement of shoot or of shoot and root give a better straight line fit when plotted against logarithm of 2,4-D concentrations between 1 and 100p.p.m. than does the root. Since it was quicker to measure the shoot alone, this was chosen for the measurement.

The comparison of germinated with ungerminated wheat seeds showed that there was no less variation in the measurements of growth in previously germinated seeds (see Table 26).

In examining the growth of ungerminated wheatseeds over two, three and four days, it was decided that two days did not allow enough time for growth, but that the results after three days were as clear and easy to measure as after four (see Figures 1 - 5).

It was decided that it was no quicker to measure seed growth in petri dishes positioned almost vertically. It was easier and more accurate to remove the seedlings from the dish to measure them than to measure them against the glass. It was also much easier to position the dishes horizontally.

Plates 1 - 4: comparison of transverse sections of wheat seedlings, three days after the ungerminated seeds had been placed on filter papers soaked in:

a) distilled water - controls.

b) 100p.p.m. 2,4-D solution.

Sections were made from segments of the root and they were photographed through the eye-piece of the microscope at eighty and three hundred and twenty magnification.

 represents .1mm on the photographs at the lower magnification.

 represents .05mm on the photographs at the higher magnification.

Plate 1: Control section of wheat root.

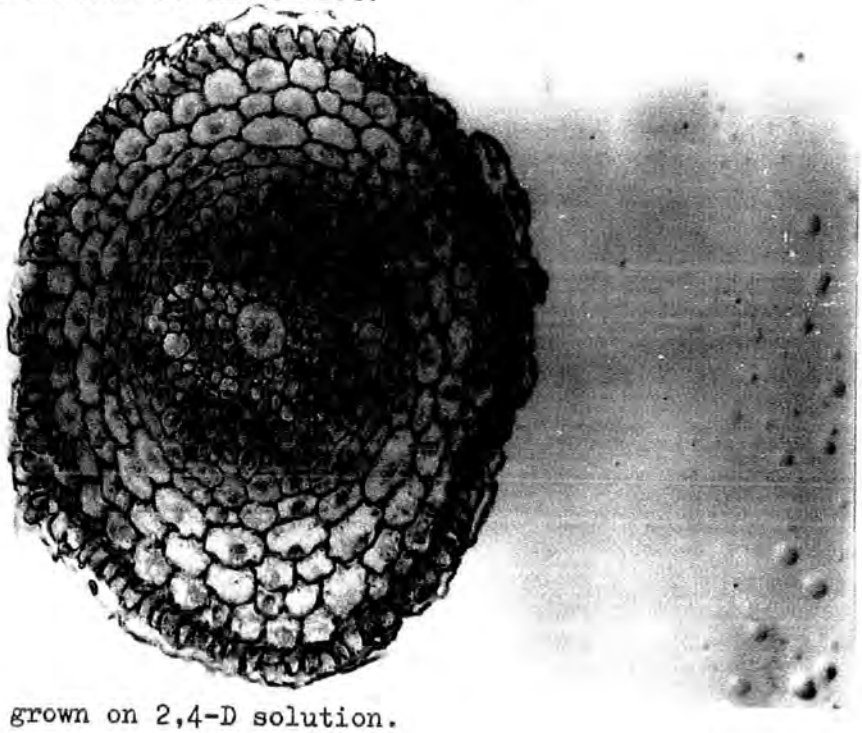


Plate 2: Section grown on 2,4-D solution.

Plate 3: Control section showing stele, endodermis and inside of cortex.

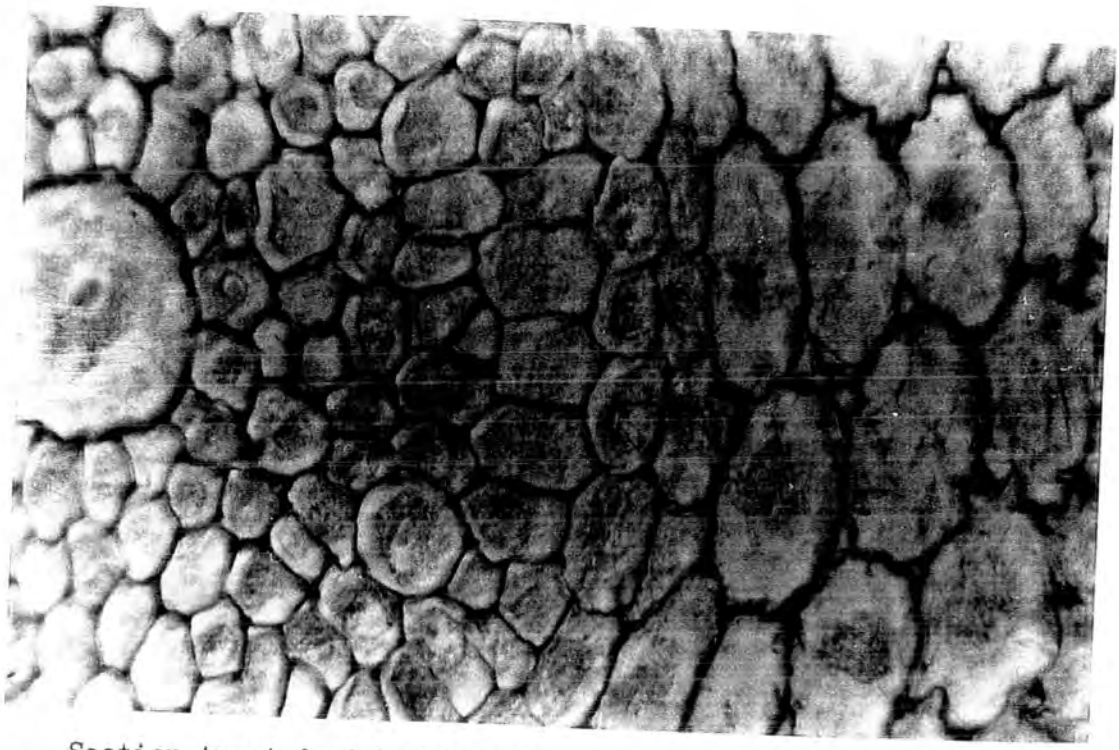


Plate 4: Section treated with 2,4-D showing endodermis and casparian strip.

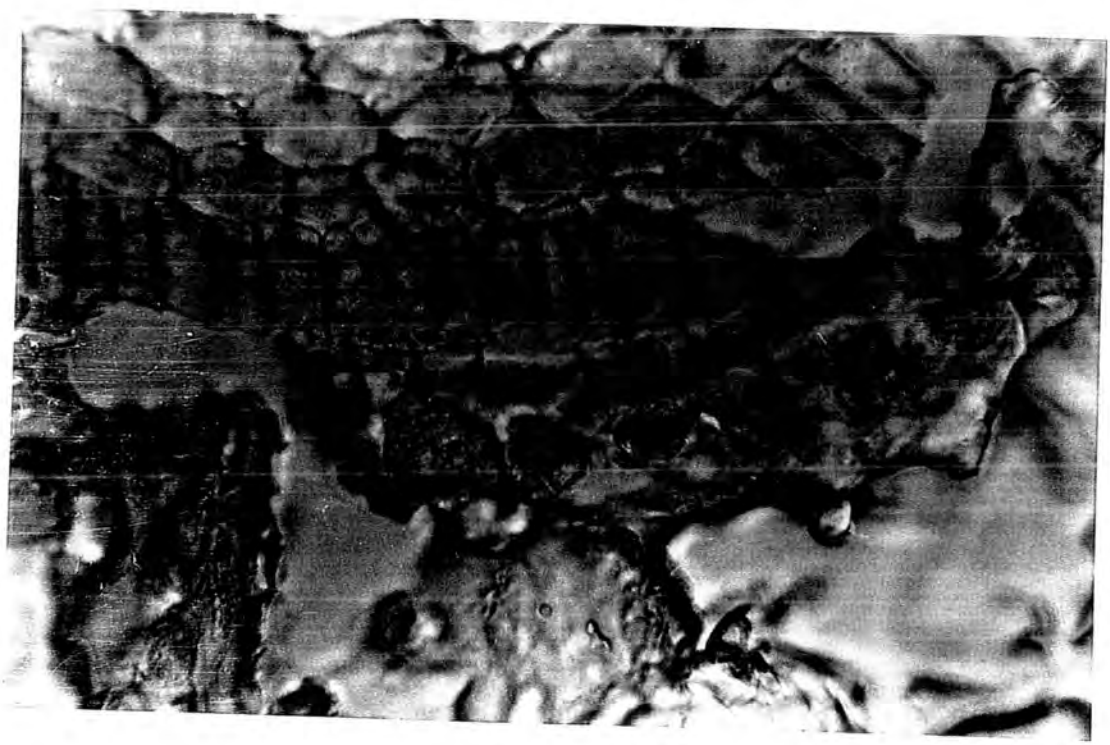


Figure 1: Comparison of three with four days growth of wheat seeds - Thrislington Common bottom, middle and top soils.

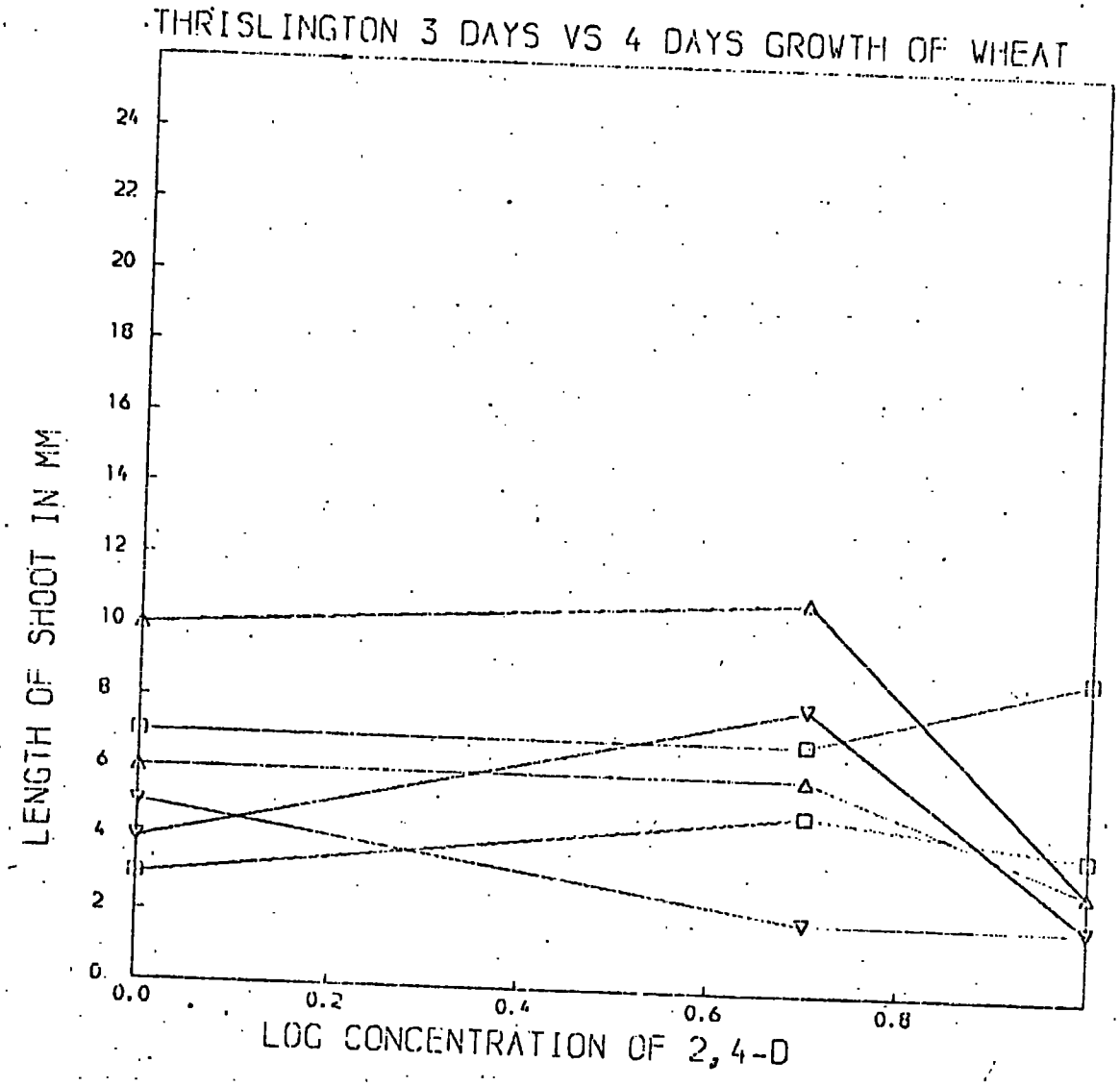

THRISLINGTON 3 DAYS VS 4 DAYS GROWTH OF WHEAT
LENGTH OF SHOOT IN MM
0
2
4
6
8
10
12
14
16
18
20
22
24
0.0
0.2
0.4
0.6
0.8
LOG CONCENTRATION OF 2,4-D

Figure 2: Comparison of three with four days growth of wheat seeds - turf bottom, middle and top soils.

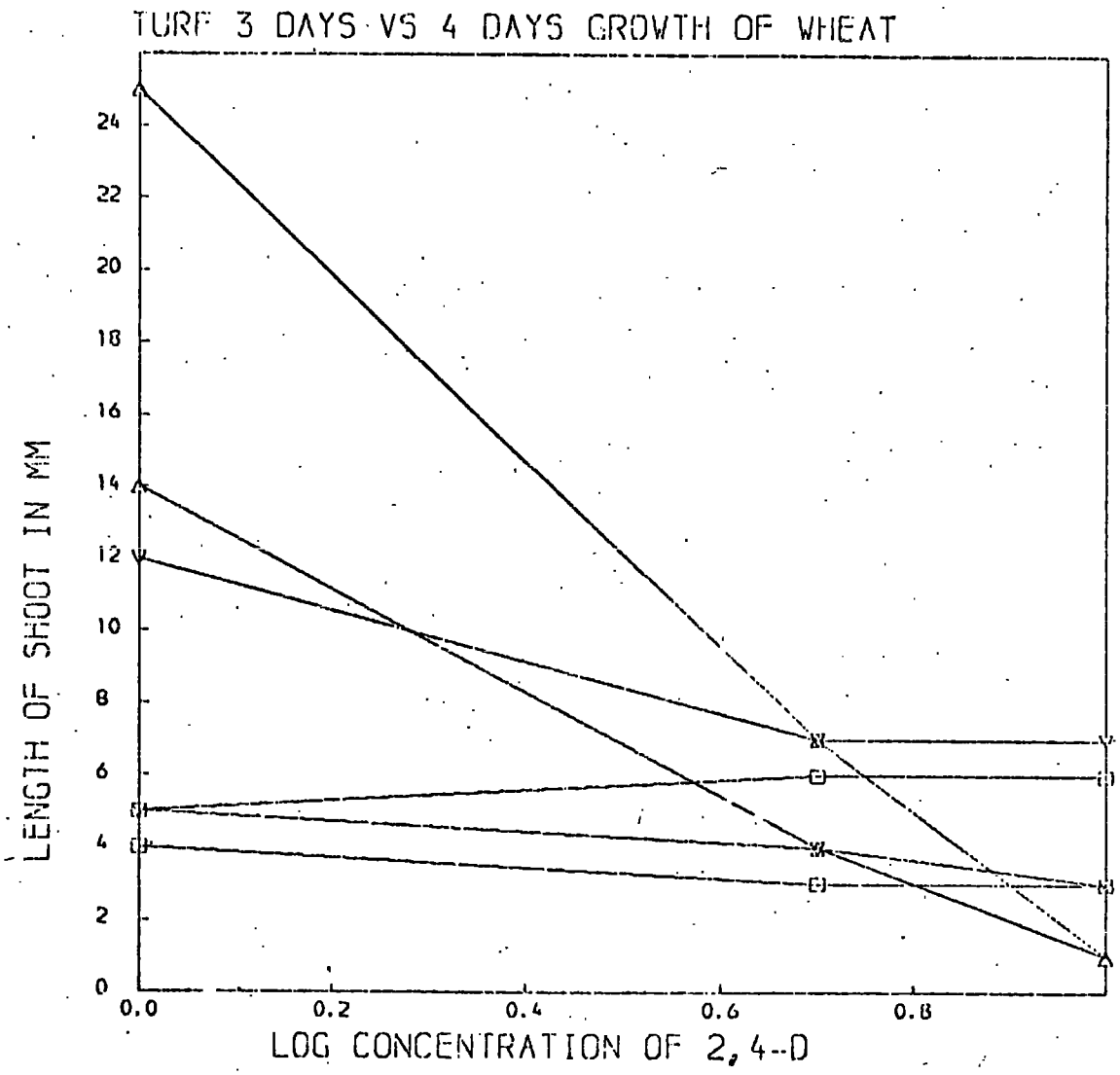

TURF 3 DAYS VS 4 DAYS GROWTH OF WHEAT
LENGTH OF SHOOT IN MM
0
2
4
6
8
10
12
14
16
18
20
22
24
0.0
0.2
0.4
0.6
0.8
LOG CONCENTRATION OF 2,4-D

Figure 3: comparison of three with four days growth of wheat seeds - wood bottom, middle and top soils.

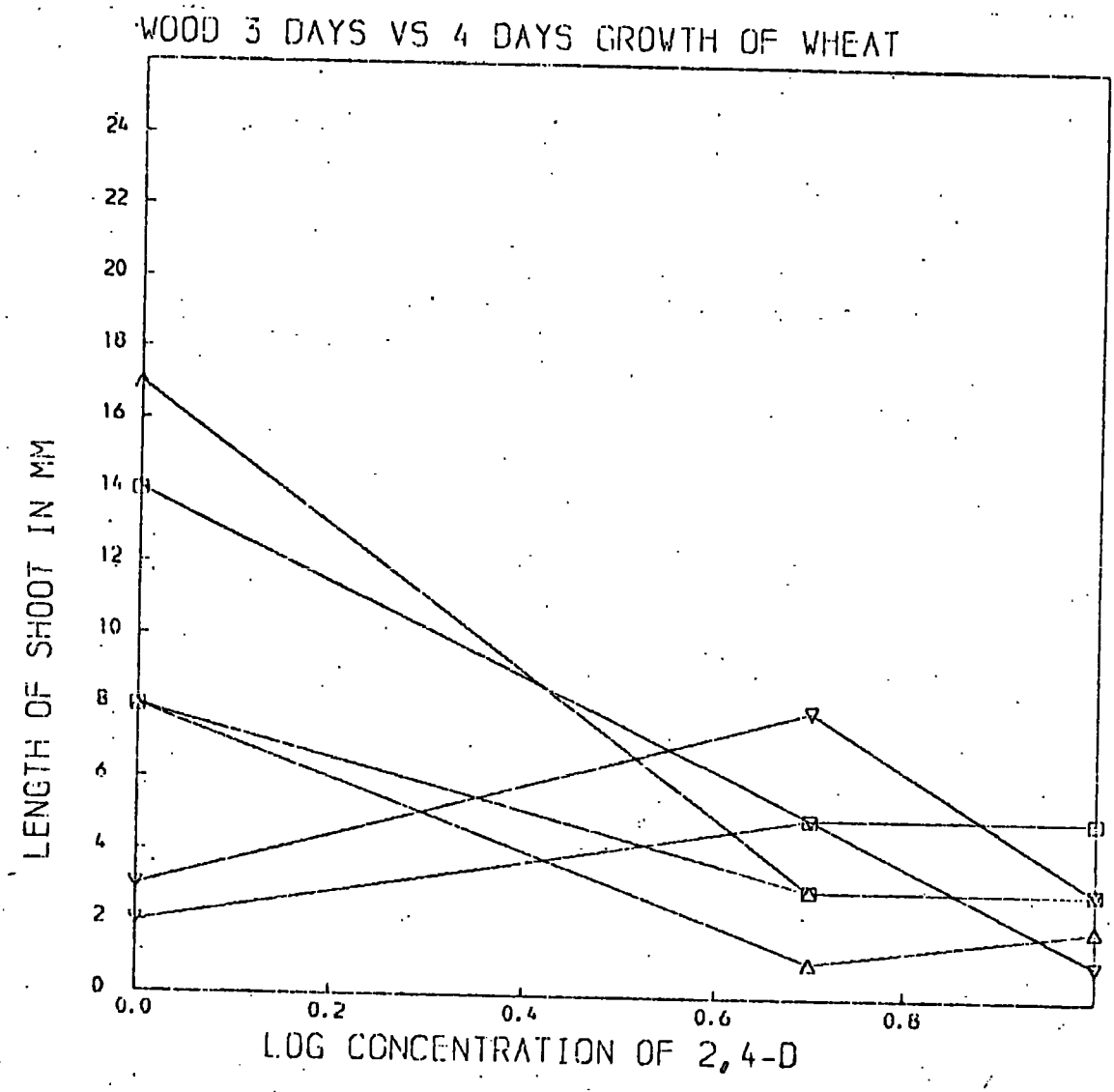

WOOD 3 DAYS VS 4 DAYS GROWTH OF WHEAT
LENGTH OF SHOOT IN MM
0
2
4
6
8
10
12
14
16
18
20
22
24
0.0
0.2
0.4
0.6
0.8
LOG CONCENTRATION OF 2,4-D

Figure 4: comparison of three with four days growth of wheat seeds - loam bottom, middle and top soils.

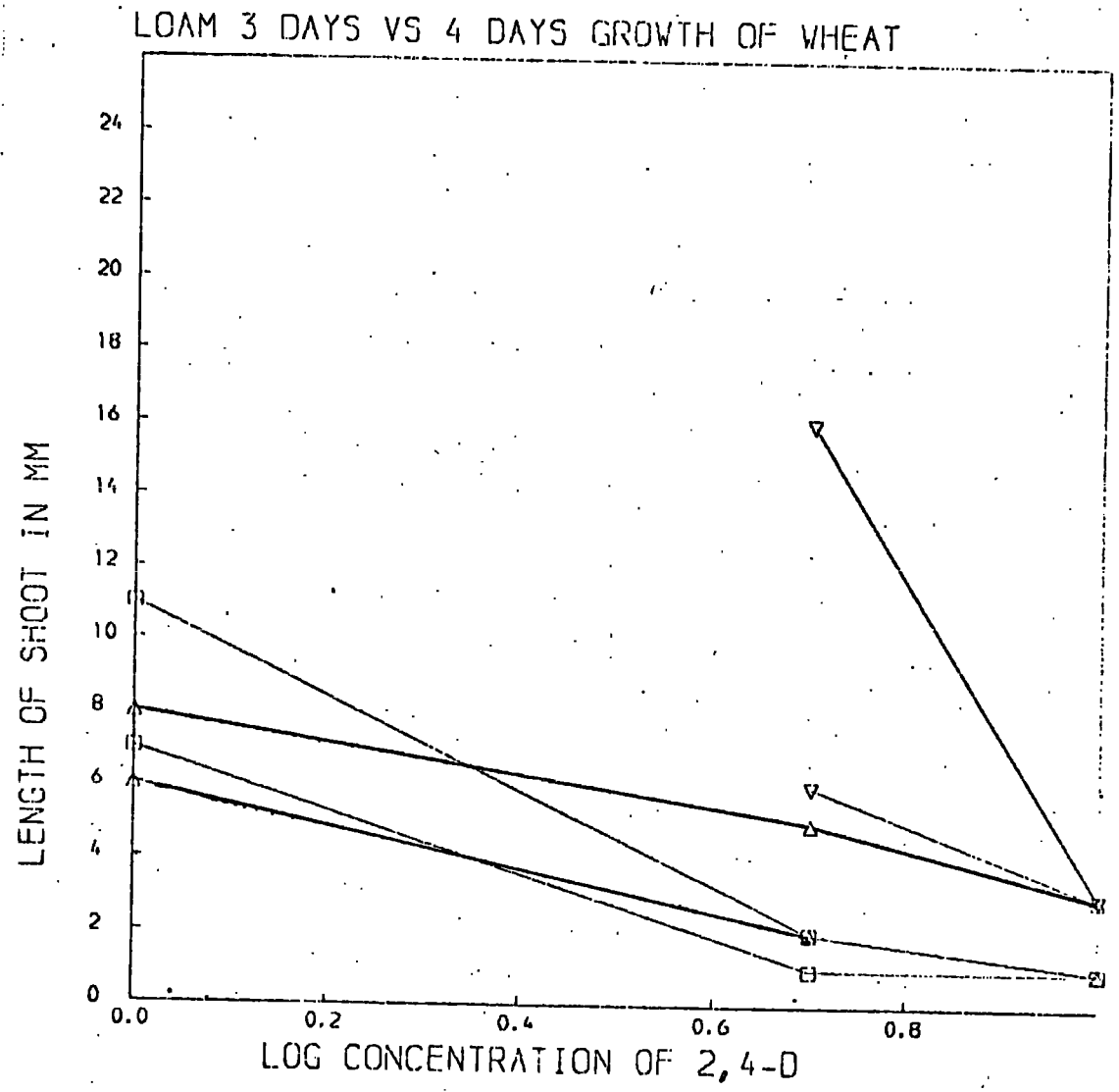
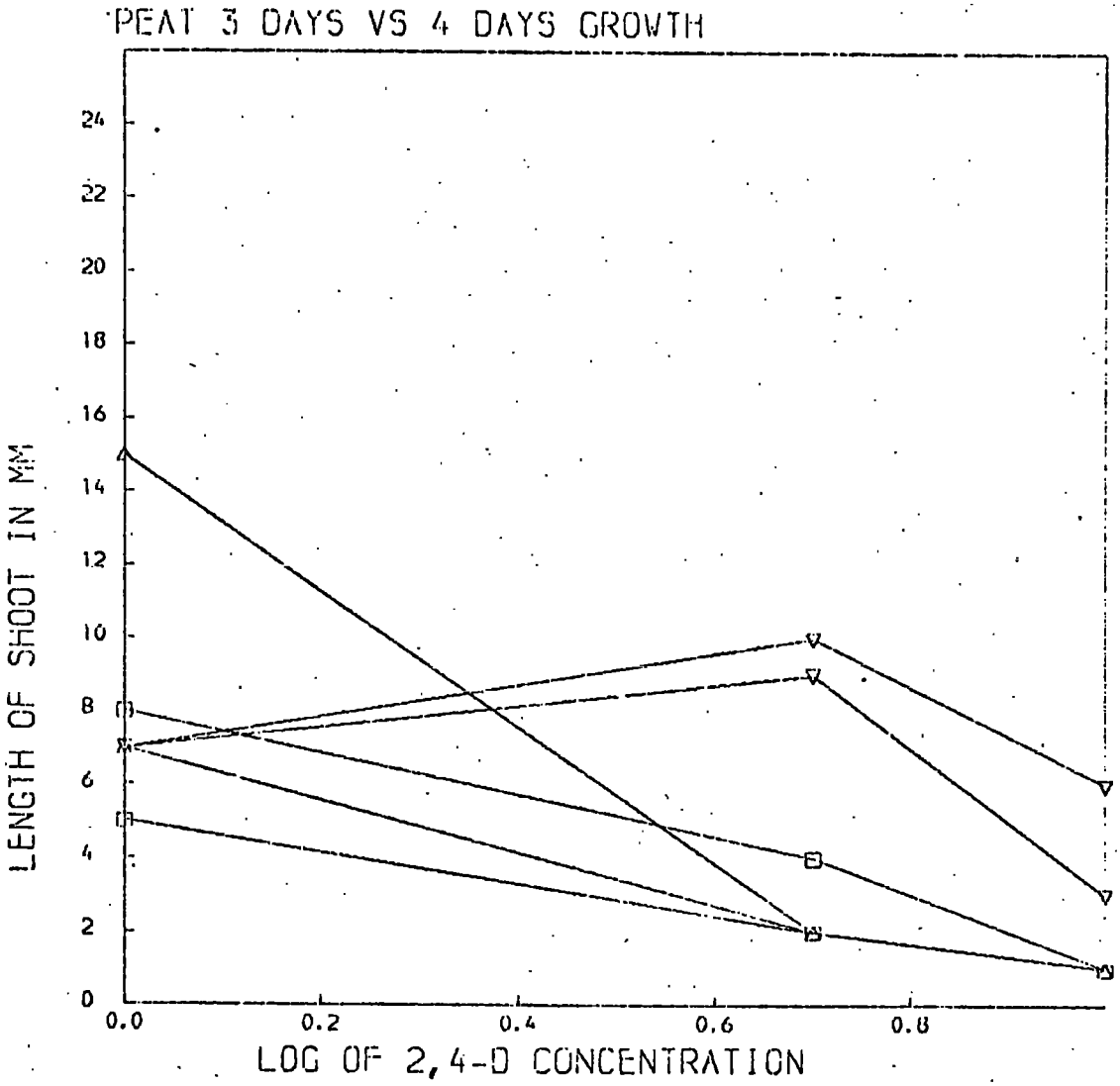

LOAM 3 DAYS VS 4 DAYS GROWTH OF WHEAT
LENGTH OF SHOOT IN MM
0
2
4
6
8
10
12
14
16
18
20
22
24
0.0
0.2
0.4
0.6
0.8
LOG CONCENTRATION OF 2,4-D

Figure 5: comparison of three with four days growth of wheat seeds - peat bottom, middle and top soils.


PEAT 3 DAYS VS 4 DAYS GROWTH
LENGTH OF SHOOT IN MM
0
2
4
6
8
10
12
14
16
18
20
22
24
0.0
0.2
0.4
0.6
0.8
LOG OF 2,4-D CONCENTRATION

3. Bioassays:

The following graphs (figures 6-20), show the control graphs which were used to calculate the 2,4-D in each soil from the shoot index calculated from each bioassay. The line plotted is the least squares fit for a straight line, and its significance is given in table 10. As can be seen from this, four of the soils (Thrislington Common middle and top, and peat middle and top) have significances below 0.1. The graphs for peat middle and peat top have not been further used. Turf bottom, turf middle and wood middle have significances of only 0.1; so, although these graphs and those of Thrislington Common middle and top have been used where they give reasonable results, the results thus obtained should be regarded with suspicion.

TABLE 10:

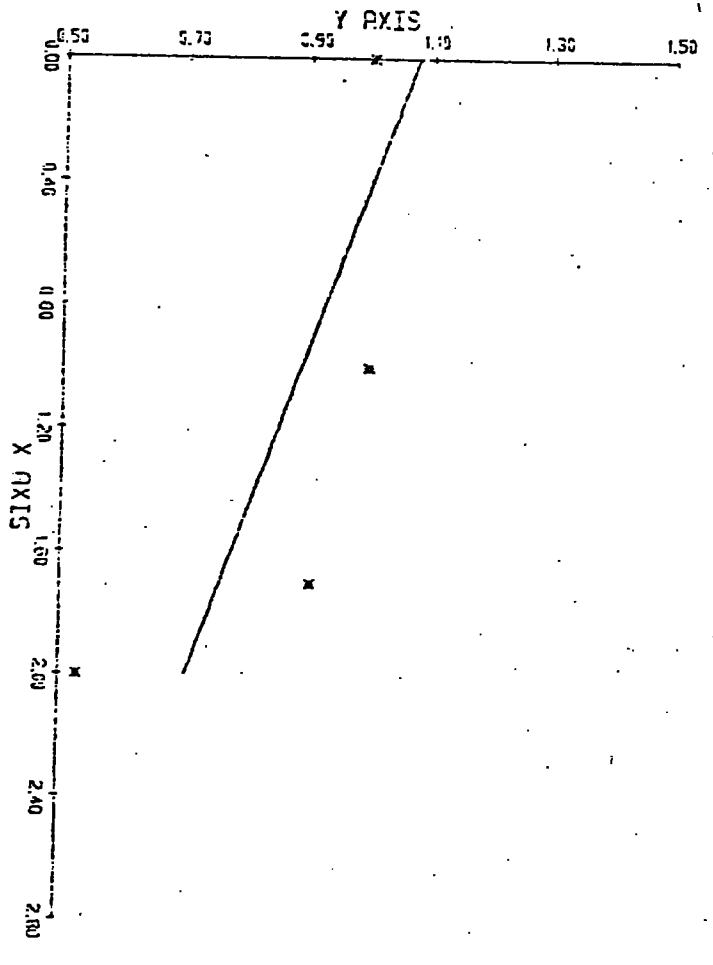
Calculation of log concentrations of 2,4-D from shoot indices was carried out using the following control graphs, where x is the logarithm of 2,4-D concentration in p.p.m. and y is the shoot index.

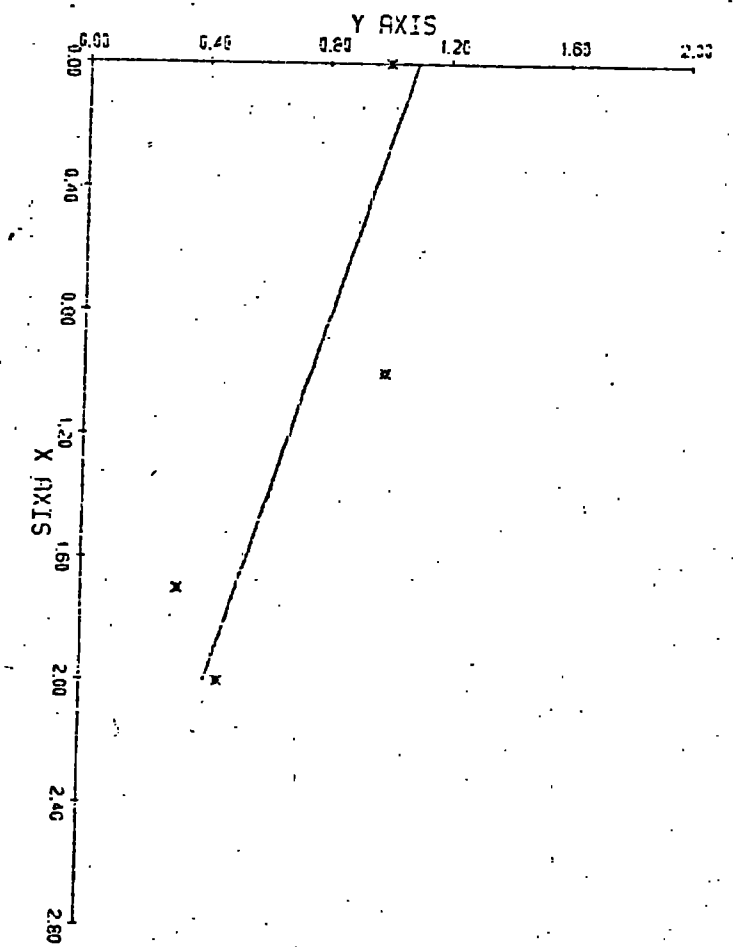
Soil type	Equation	Coefficient of correlation, r	Significance, using Student's t-test
TCB	y=.976-.367x	.99	.02
TCM	y=1.09-.339x	.84	< .1
TCT	y=1.08-.184x	.72	< .1
TB	y=1.09-.428x	.94	.1
TM	y=.967-.404x	.94	.1
TT	y=.971-.401x	.99	.02
WB	y=.976-.457x	.99	.02
WM	y=.893-.364x	.90	.1
WT	y=1.05-.427x	.98	.05
LB	y=.972-.446x	.99	.01
LM	y=1.02-.381x	.97	.05
LT	y=.959-.429x	.99	.02
PB	y=.995-.255x	.97	.05
PM	y=1.01-.00333x	.62	< .1
PT	y=1.04-.116x	.88	< .1

Figure 6: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - Thrislington Common bottom.

Figure 7: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - Thrislington Common middle.

Figure 8: control graph of shoot index (7-axis) against logarithm of 2,4-D concentration (x-axis) - Thrislington Common top.


Y AXIS
0.50
0.70
0.90
1.10
1.30
1.50
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80


Y AXIS
0.00
0.40
0.80
1.20
1.60
2.00
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

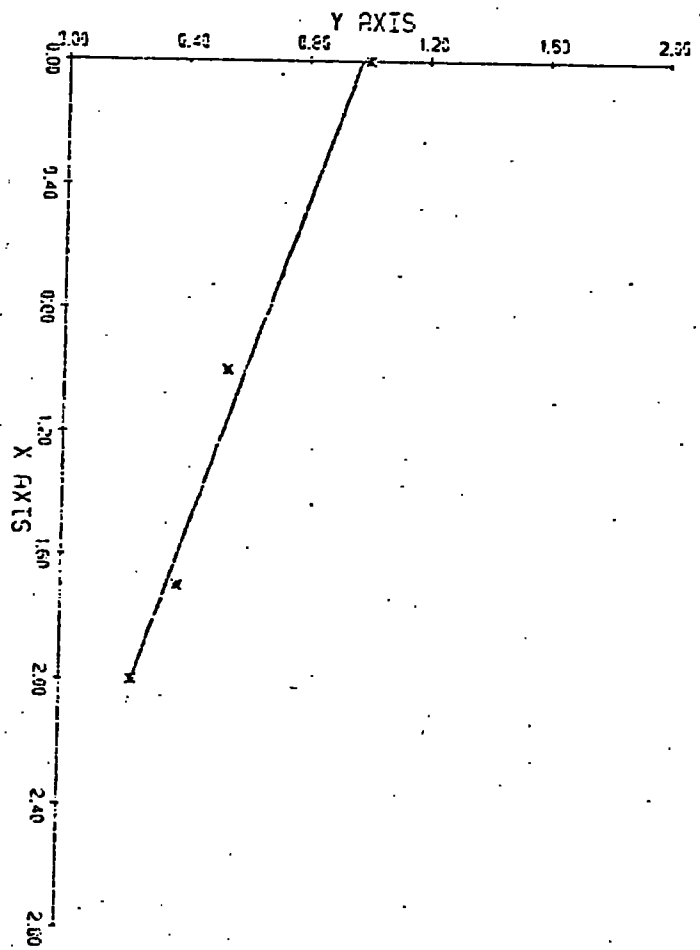
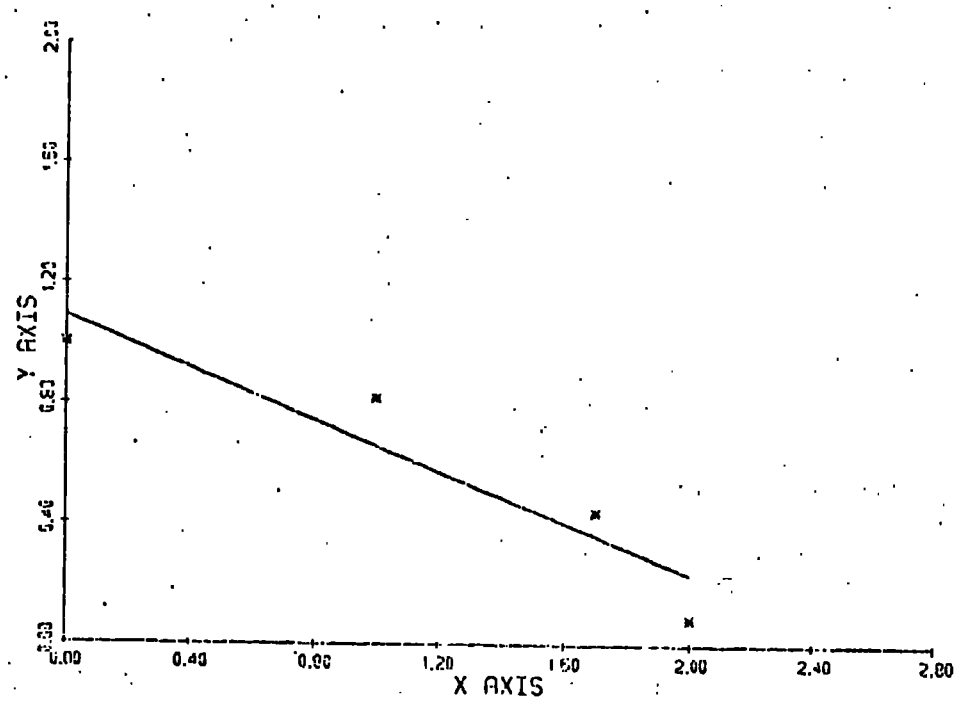
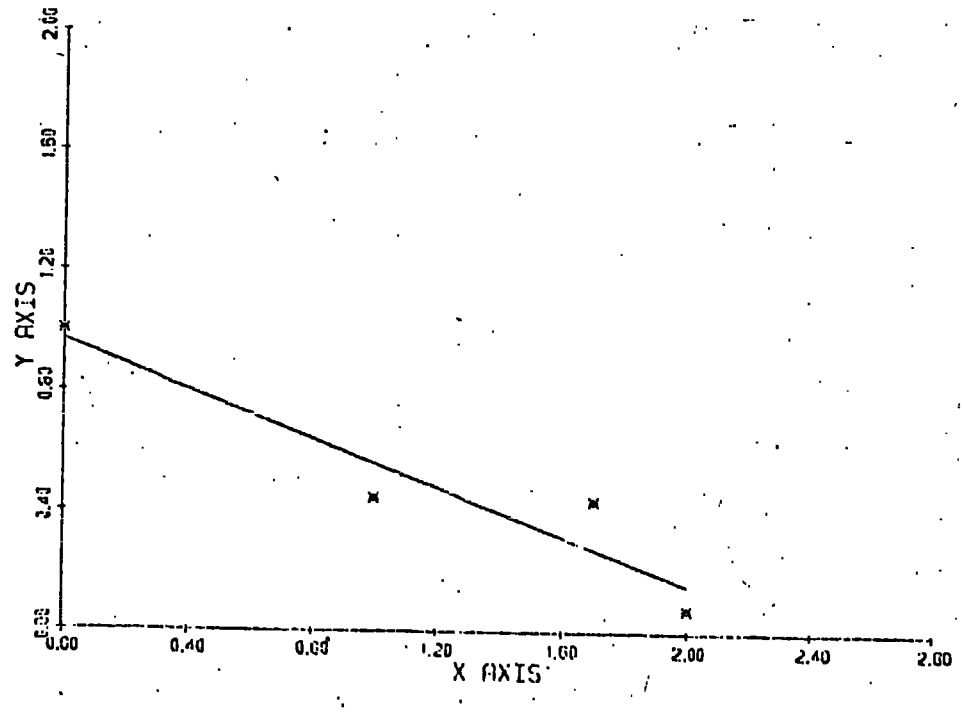

Y AXIS
0.00
0.40
0.80
1.20
1.60
2.00
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

Figure 9: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - turf bottom.

Figure 10: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - turf middle.

Figure 11: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - turf top.


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

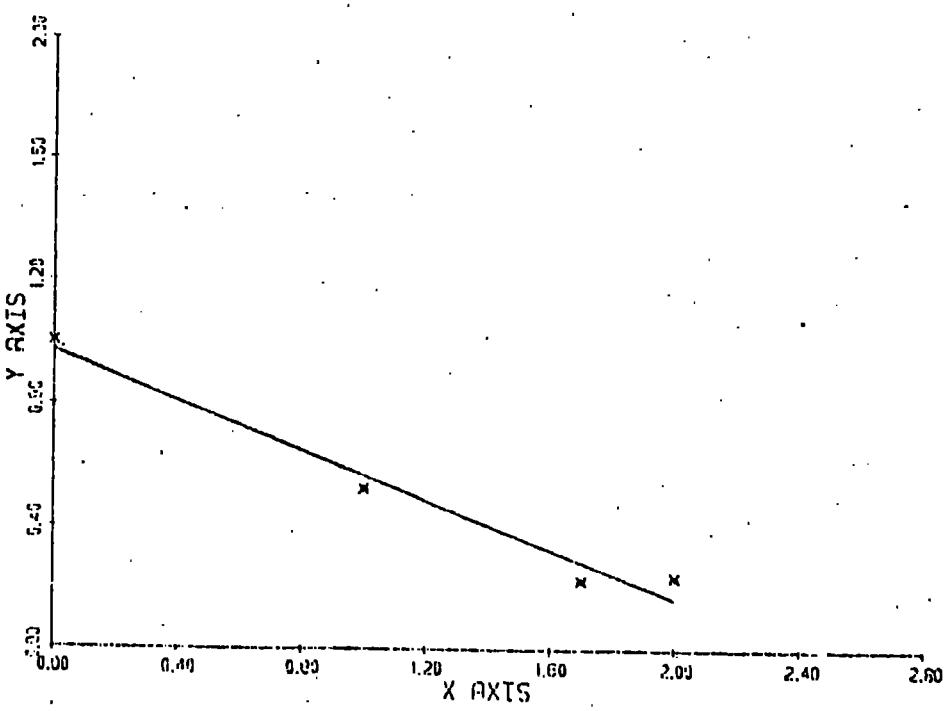
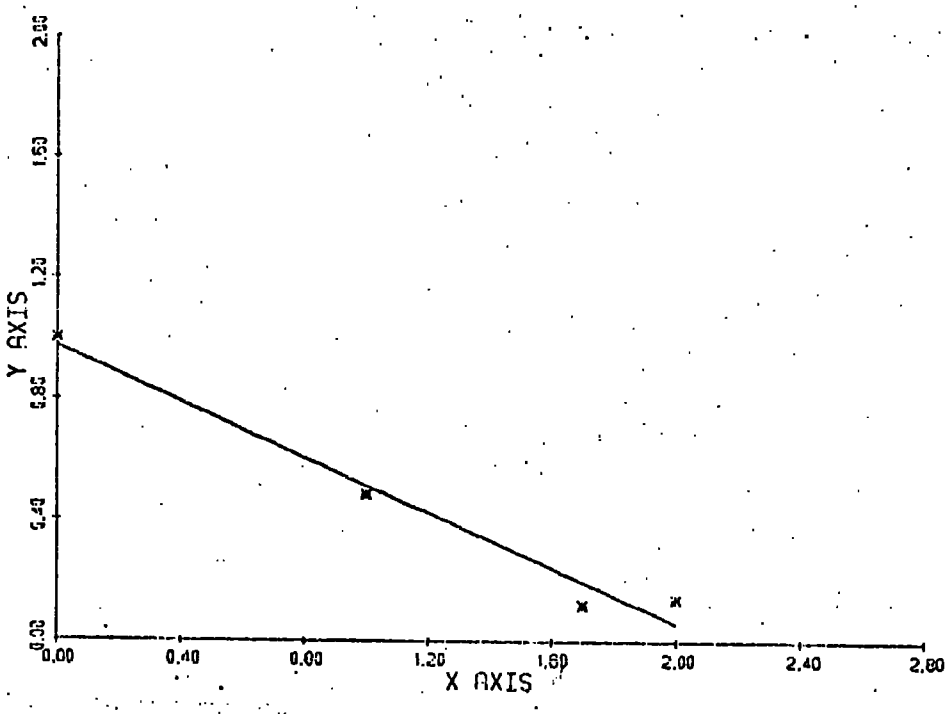
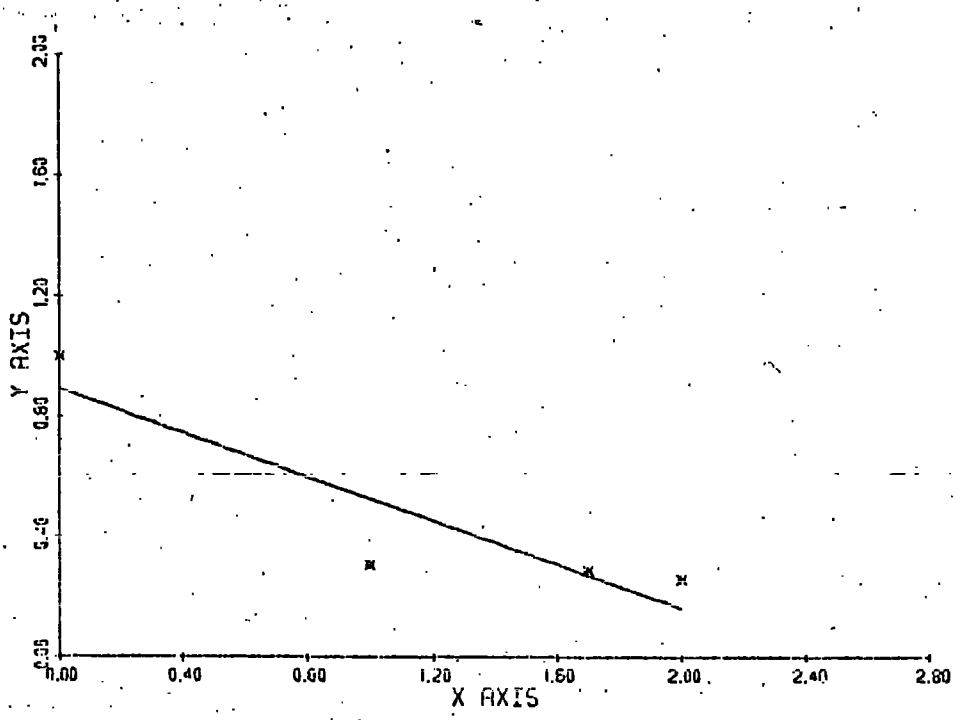

Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

Figure 12: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - wood bottom.

Figure 13: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - wood middle.

Figure 14: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - wood top.


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

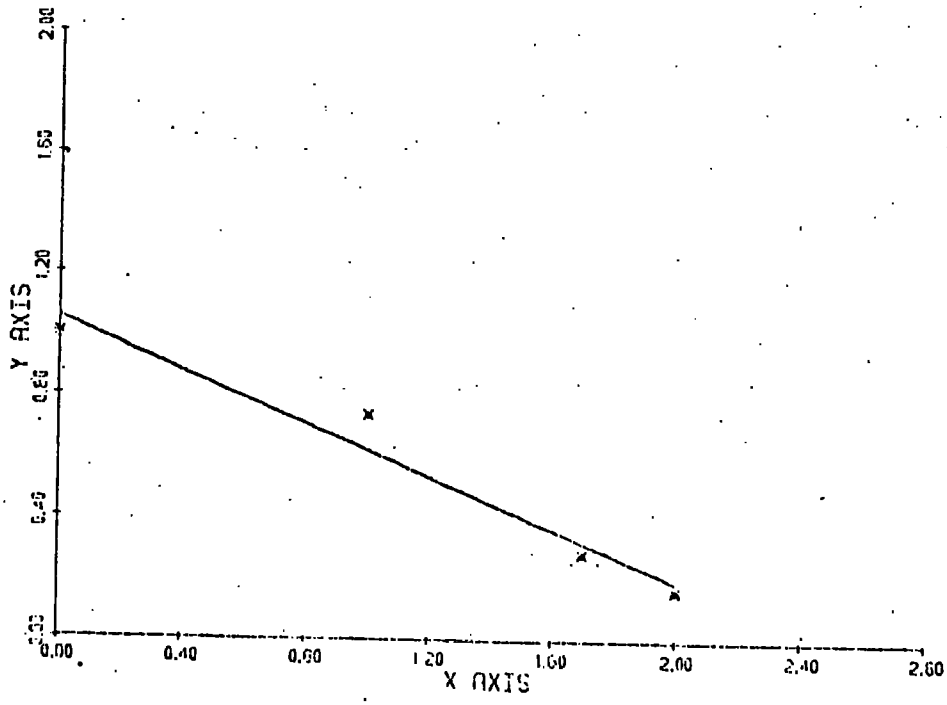
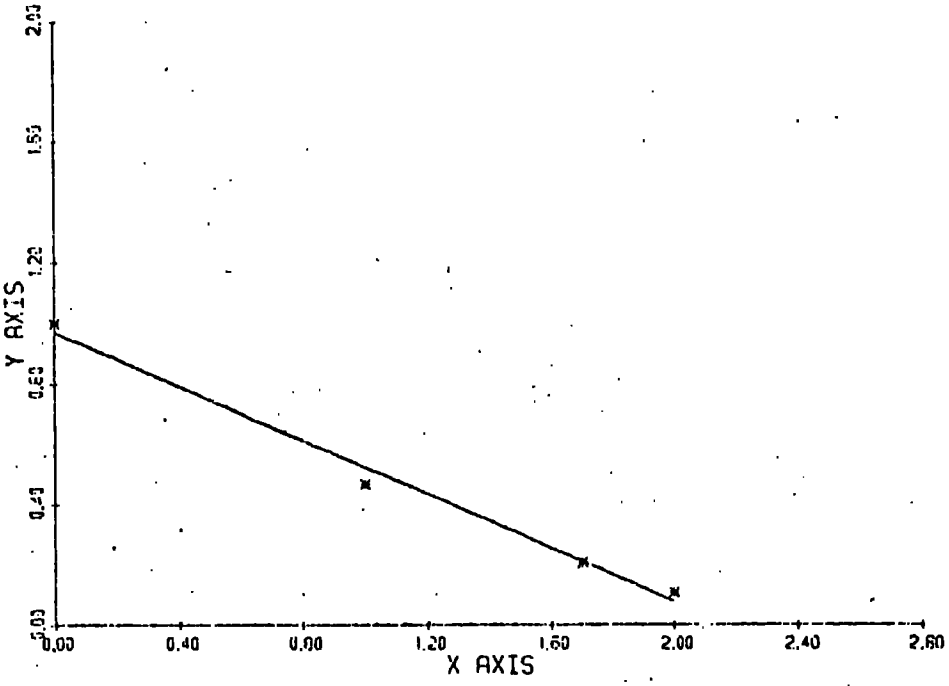
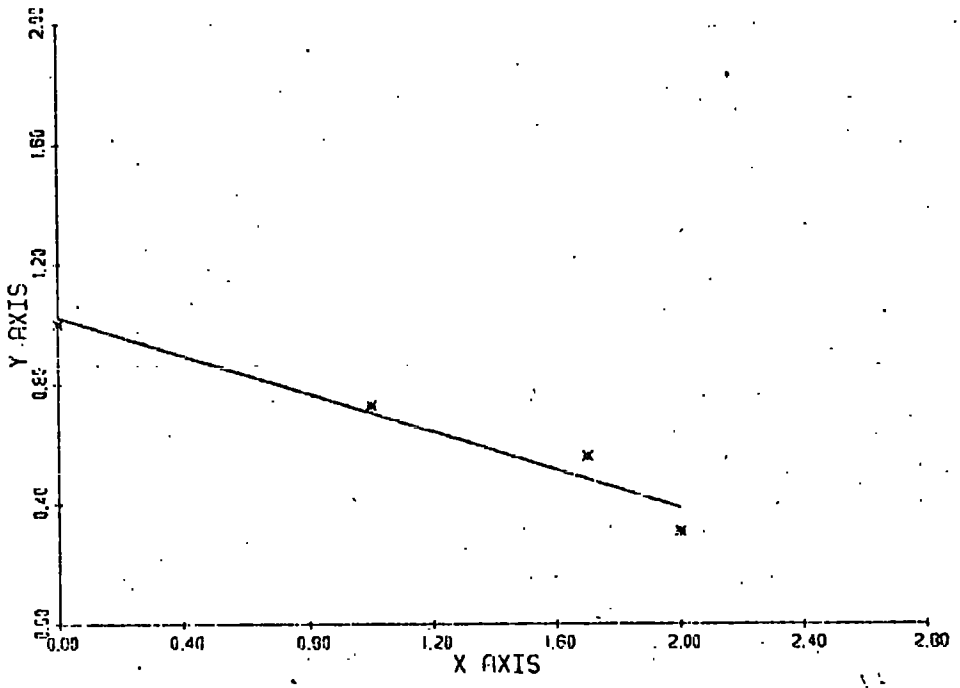

Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

Figure 15: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - loam bottom.

Figure 16: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - loam middle.

Figure 17: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - loam top.


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

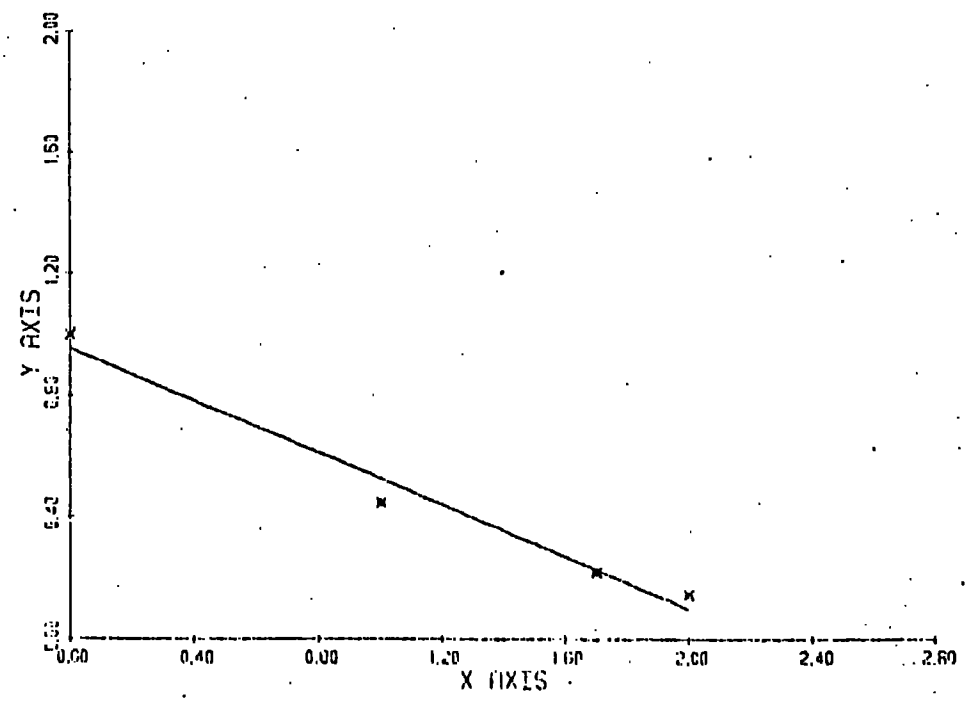
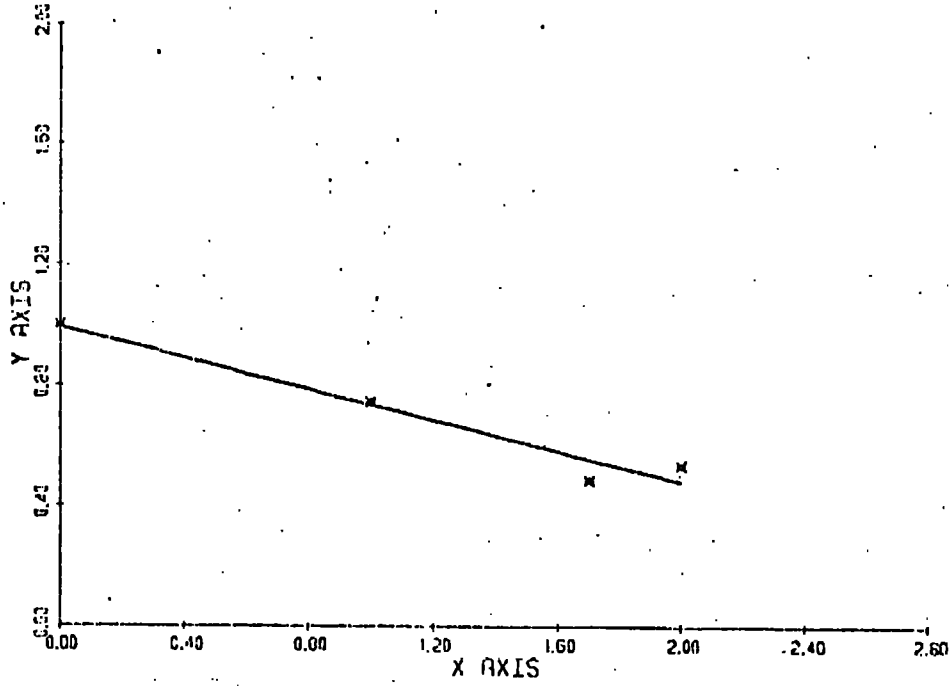
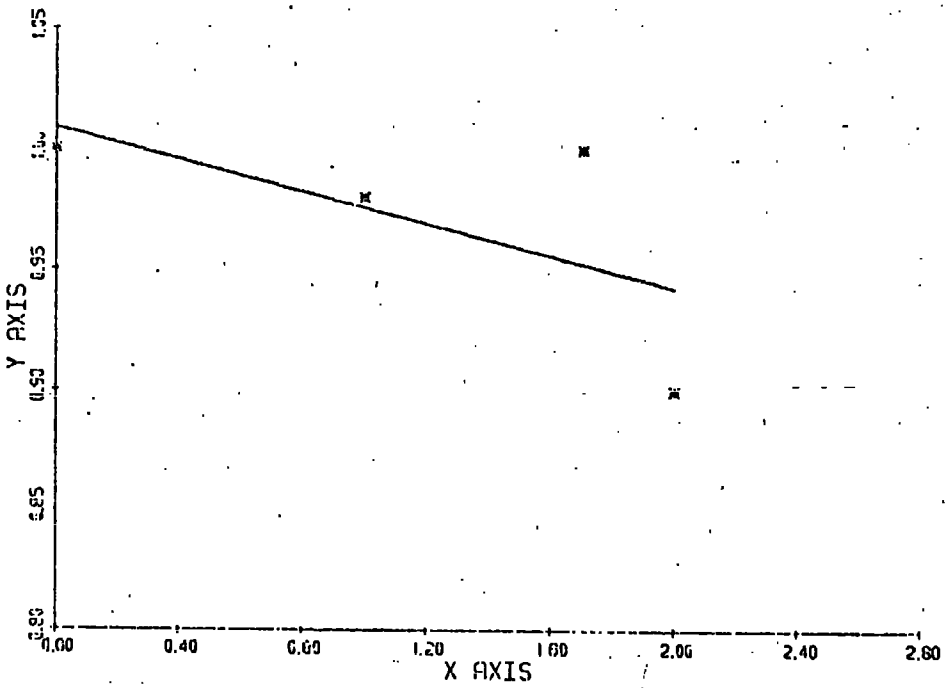

Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

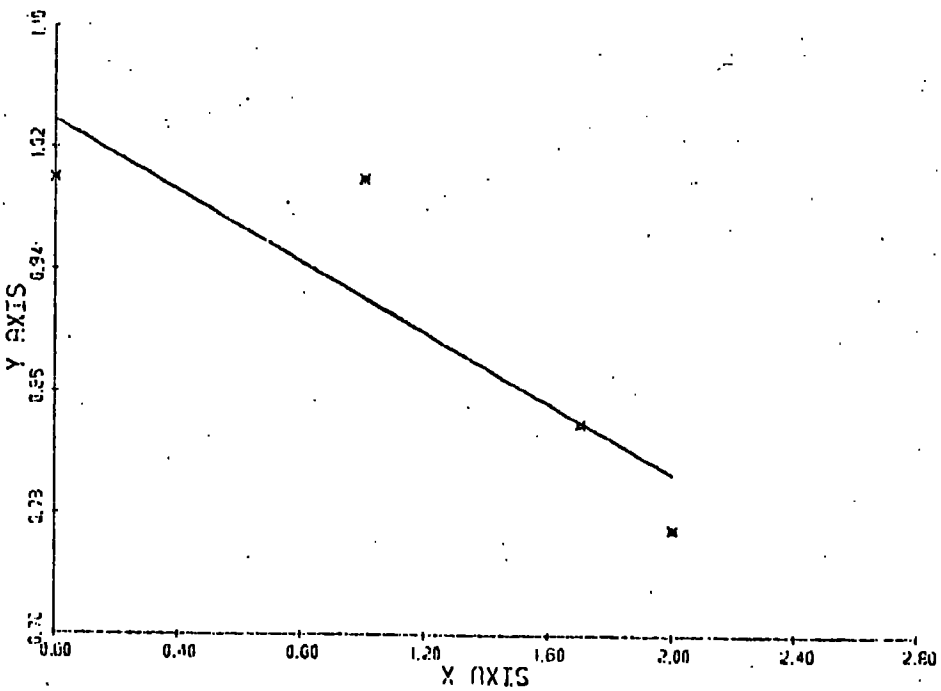
Figure 18: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - peat bottom.

Figure 19: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - peat middle.

Figure 20: control graph of shoot index (y-axis) against logarithm of 2,4-D concentration (x-axis) - peat top.


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80


Y AXIS
X AXIS
0.00
0.40
0.80
1.20
1.60
2.00
2.40
2.80

Weekly bioassays were carried out on the following dates:

	Date bioassay started
Week 0	25th June
Week 1	2nd July
Week 2	9th July
Week 3	16th July
Week 4 (also Week 0)	23rd July
Week 5 (also Week 1)	30th July
Week 6 (also Week 2)	6th August
Week 7 (also Week 3)	12th August

Abbreviations

TC	Thrislington Common
T	Turf
W	Wood
L	Loam
P	Peat.
B	Bottom soil
M	Middle soil
T	Top soil

Figures 21-25 show the results of calculating 2,4-D concentrations for the weekly bioassays by dividing the weekly mean of shoot length for each soil by the mean of the shoot length of the original controls, to obtain the shoot index. The symbols used for each soil are explained on page 29.

The persistence in weeks as calculated from these graphs (see Chapter 4) is given in table 11. These values are used to attempts to find any correlations with soil properties, and the results given in table 12.

In figures 26-28, the 2,4-D concentrations have been calculated using the weekly controls set up at the same time as the bioassays. Soils have not been included in the graph where results for 2,4-D concentration have been outside the range under consideration (1 - 100p.p.m.), as this is probably caused by lack of significance in the relevant control graphs (figures 6-20).

In figures 29 and 30, the wet and dry soils from Thrislington Common, studied over three weeks, are compared. The original controls are used to obtain shoot indices. (The results obtained using weekly controls for the same set of data are shown in figures 37 and 38 in Appendix 3 .

Table 13 gives the results of the field experiments on Thrislington Common, dividing the growth in cms of wheat on soil from treated quadrats by the growth on soil from unsprayed controls to obtain a shoot index.

Table 14 shows the growth, in centimetres, of wheat shoots on filter papers soaked in the 2,4-D solution left in containers when the original bioassay experiment was set up.

4. Radio-chemical experiments:

Figure 31 shows the very marked difference in metabolic uptake of 2,4-D by wheat seeds on soil from Thrislington Common and peat treated with a solution of radioactive 2,4-D. Uptake is compared in counts per minute. The experiment was continued over three days (the same duration as the bioassays). The amount taken up by dead seeds was subtracted from that taken up by live, to obtain an estimate of metabolic uptake.

In table 15 the soils are compared before and after centrifuging. The result for sample A is very unlike all the others, and this may be due to the addition of slightly too much water in proportion to the scintillation fluid. Table 16 shows the percentages of 2,4-D still left in the germinated seeds after shaking with water.

Figure 21: weekly bioassays using original controls for calculations - Thrislington Common bottom, middle, top.

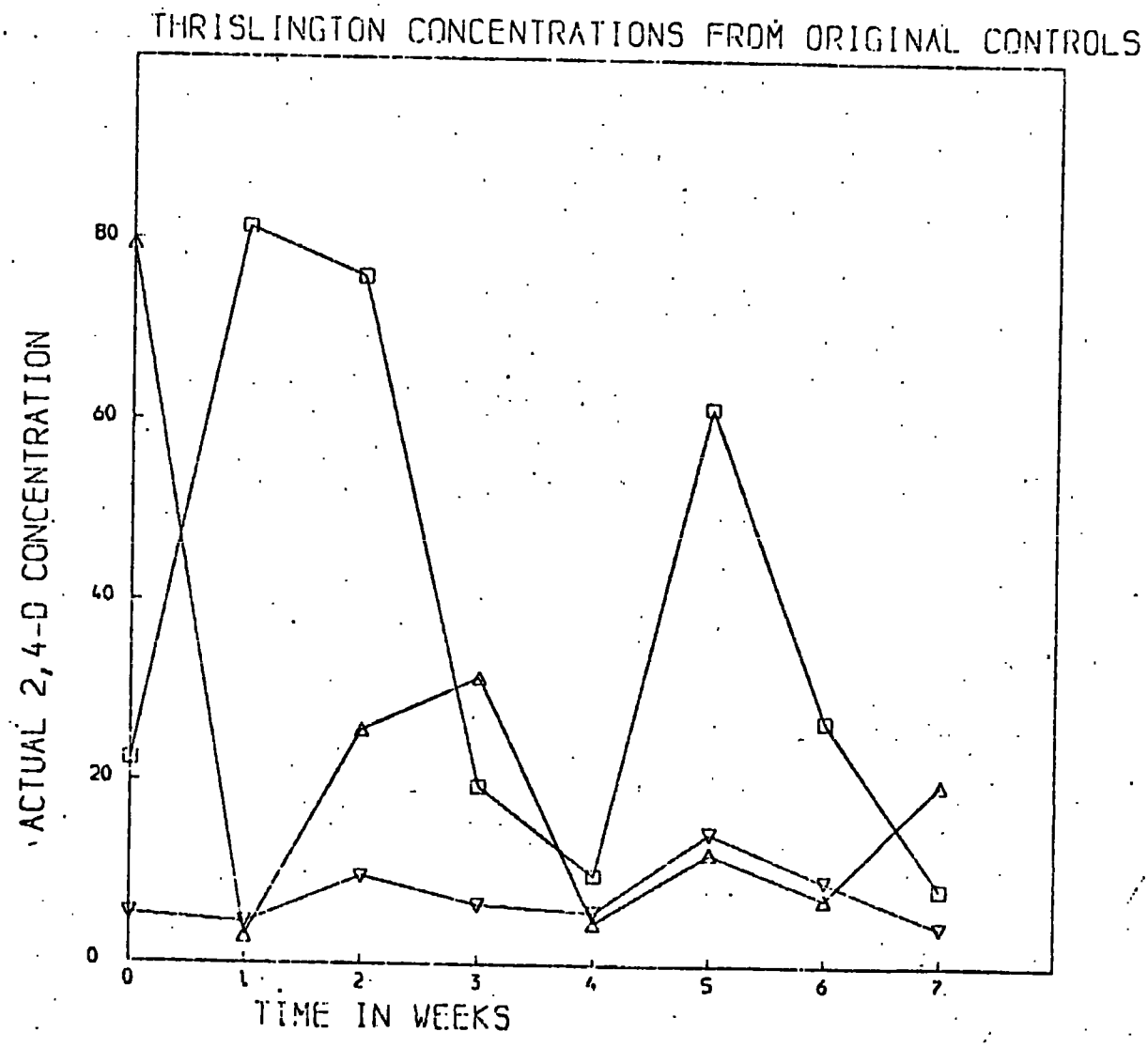

THRISLINGTON CONCENTRATIONS FROM ORIGINAL CONTROLS
ACTUAL 2,4-D CONCENTRATION
80
60
40
20
0
0
1
2
3
4
5
6
7
TIME IN WEEKS

Figure 22: weekly bioassays using original controls for calculations - turf bottom, middle, top.

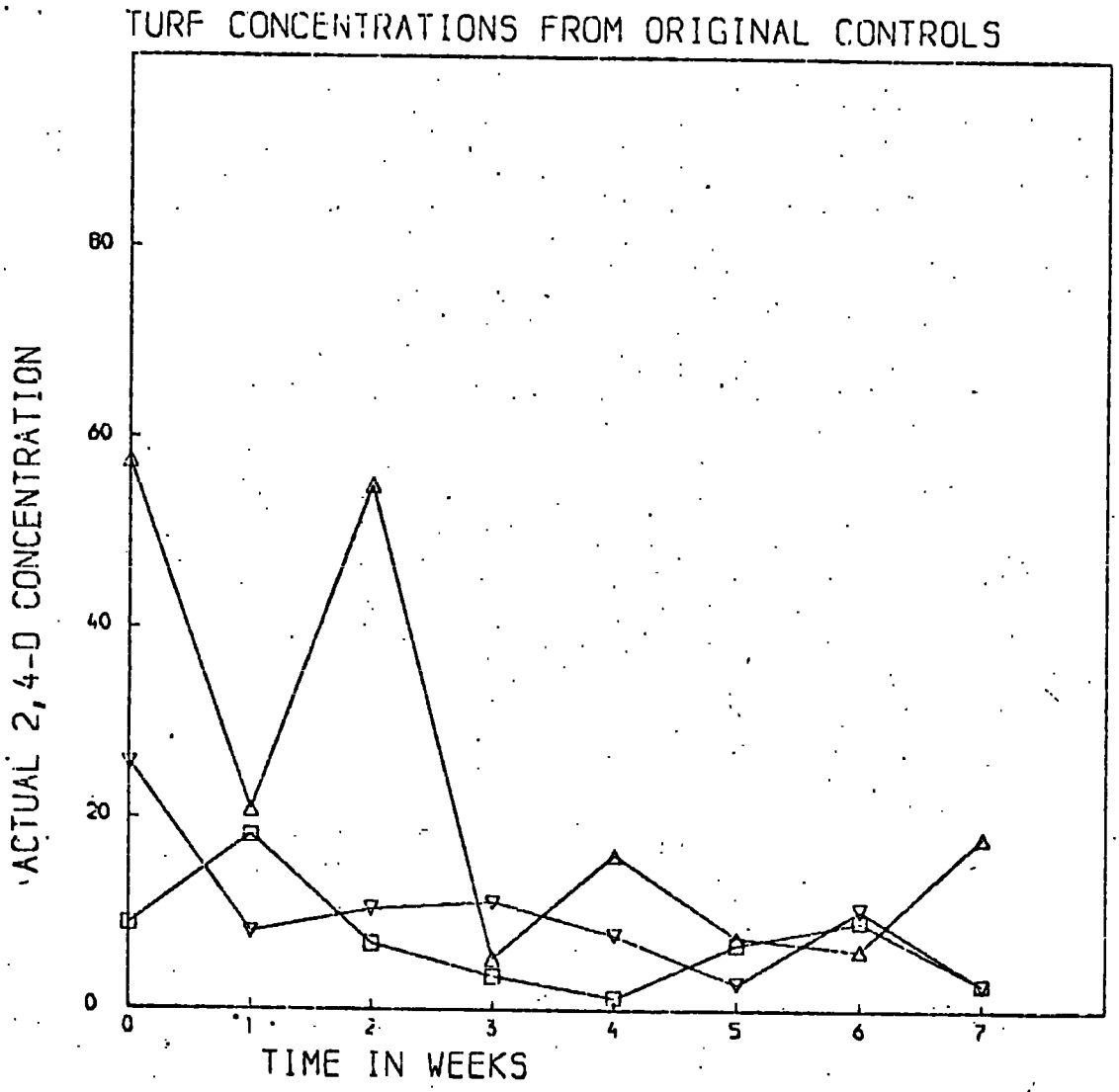

TURF CONCENTRATIONS FROM ORIGINAL CONTROLS
ACTUAL 2,4-D CONCENTRATION
80
60
40
20
0
0
1
2
3
4
5
6
7
TIME IN WEEKS

Figure 23: weekly bioassays using original controls for calculations - wood bottom, middle, top.

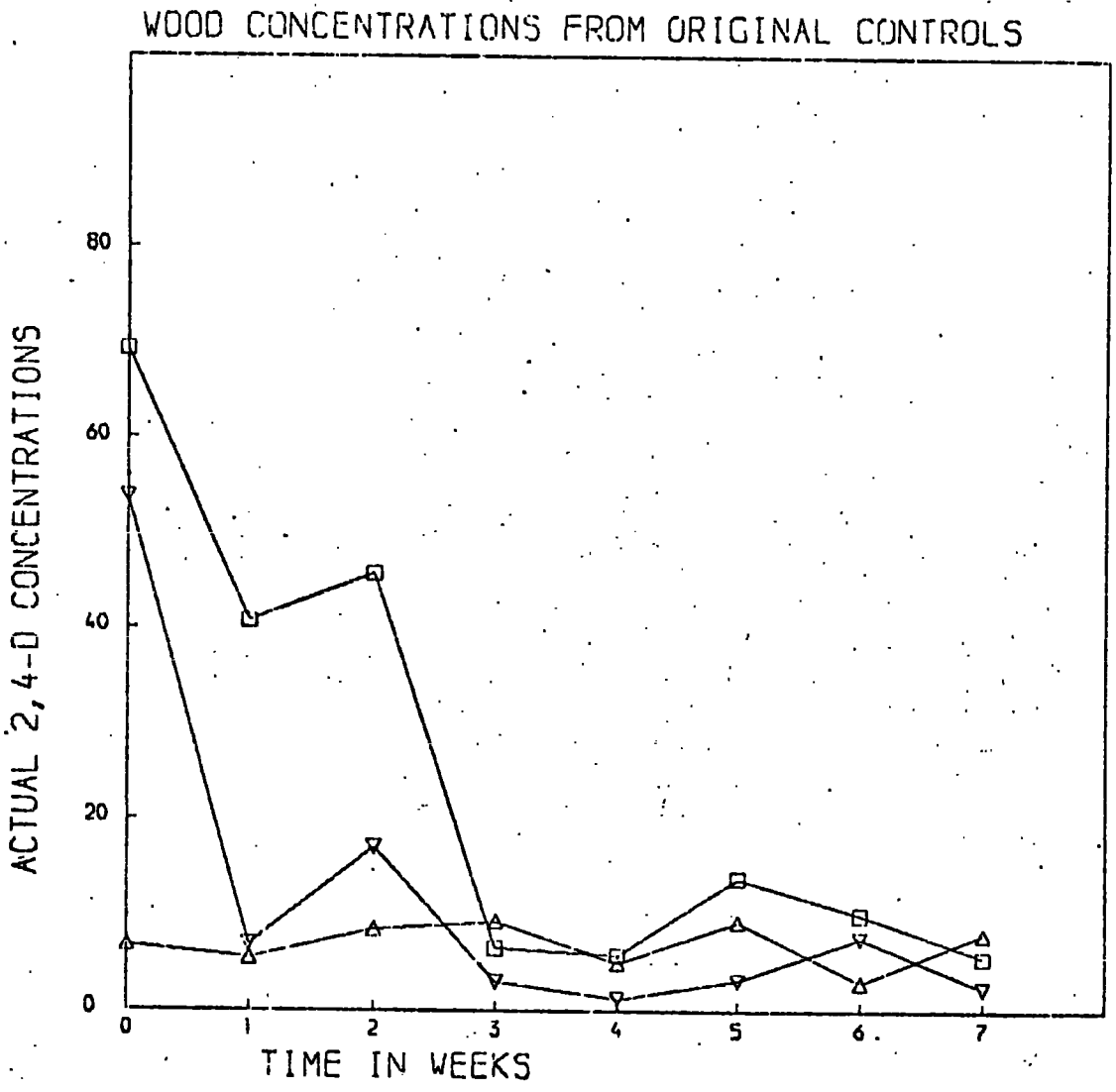

WOOD CONCENTRATIONS FROM ORIGINAL CONTROLS
ACTUAL 2,4-D CONCENTRATIONS
80
60
40
20
0
0
1
2
3
4
5
6.
7
TIME IN WEEKS

Figure 24: weekly bioassays using original controls for calculations - loam bottom, middle, top.

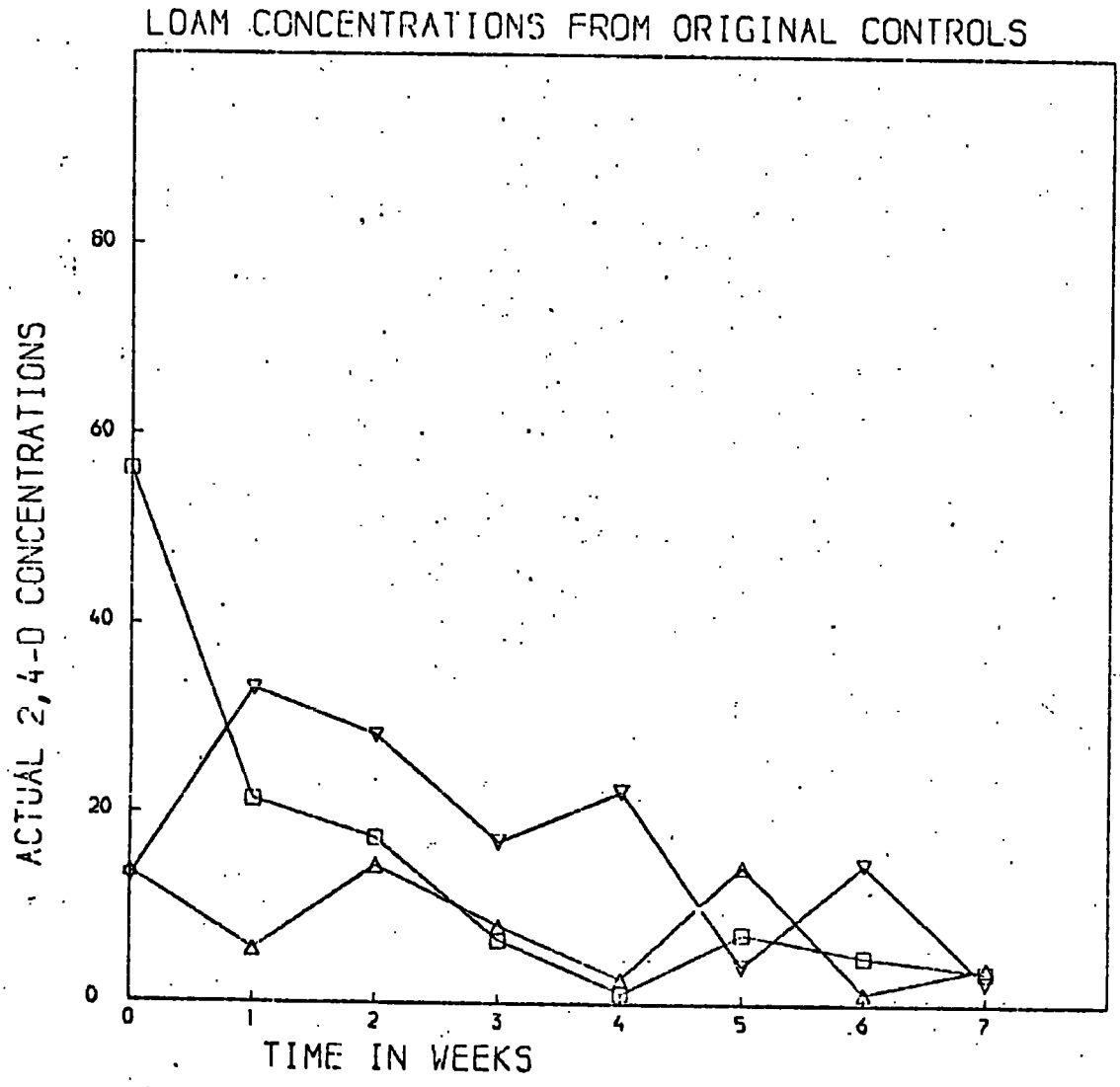

LOAM CONCENTRATIONS FROM ORIGINAL CONTROLS
ACTUAL 2,4-D CONCENTRATIONS
80
60
40
20
0
0
1
2
3
4
5
6
7
TIME IN WEEKS

Figure 25: weekly bioassays using original controls for calculations - peat bottom, middle, top.

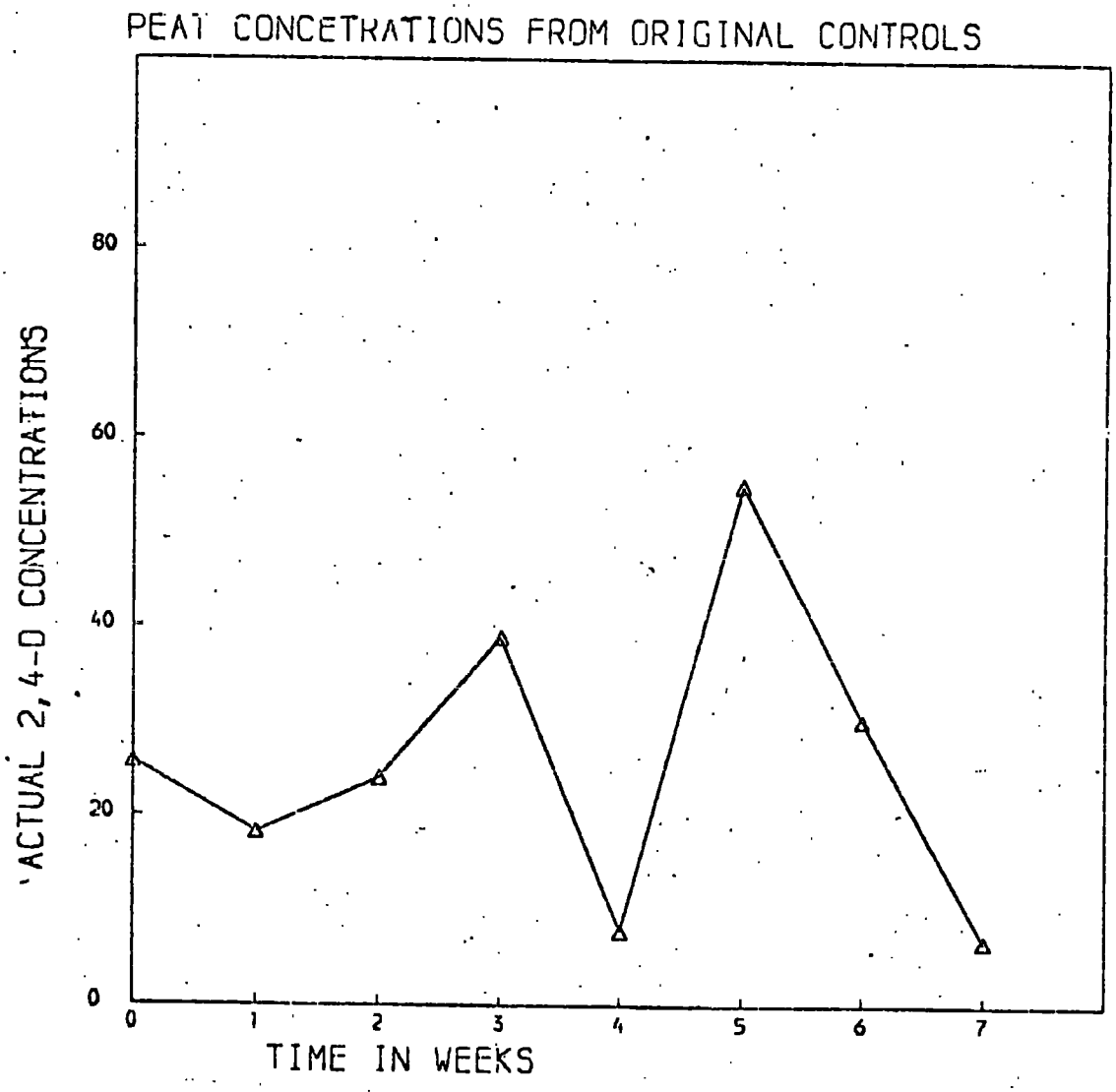

PEAT CONCETRATIONS FROM ORIGINAL CONTROLS
ACTUAL 2,4-D CONCENTRATIONS
0
20
40
60
80
0
1
2
3
4
5
6
7
TIME IN WEEKS

TABLE 11:

Weekly bioassays using original controls to assess 2,4-D concentration.

Persistence of 2,4-D

Soil type	First week that 2,4-D falls and consistently remains below 20ppm
TCB	4
TCM	0
TCT	7
TB	3
TM	1
TT	0
WB	0
WM	1
WT	3
LB	0
LM	5
LT	2
PB	7
PM	No results
PT	No results

TABLE 12:

Linear regressions and correlation coefficients obtained by plotting soil properties (y) against soil persistence of 2,4-D in weeks (x).(Peat middle and peat top are excluded, as there are no persistence results for them).

Soil property (y)	Equation of line	Correlation coefficient r
Organic carbon (peat bottom excluded since results calculated differently)	y = .09x+3.9	r = 0.21
pH	y = -.07x+6.5	r = -0.16
Cation Exchange capacity	y = 1.2x+26.6	r = 0.58
Exchangeable calcium	y = -.14x+7.9	r = -0.12
Exchangeable magnesium	y = .09x+1.4	r = 0.16
Percentage sand	y = 2.8x+36.5	r = 0.39
Percentage clay	y = -.89x+13.4	r = -0.26
Percentage silt	y = -1.9x+50.0	r = -0.47

Figure 26: weekly bioassays using weekly controls for calculations - Thrislington Common bottom, middle, top.

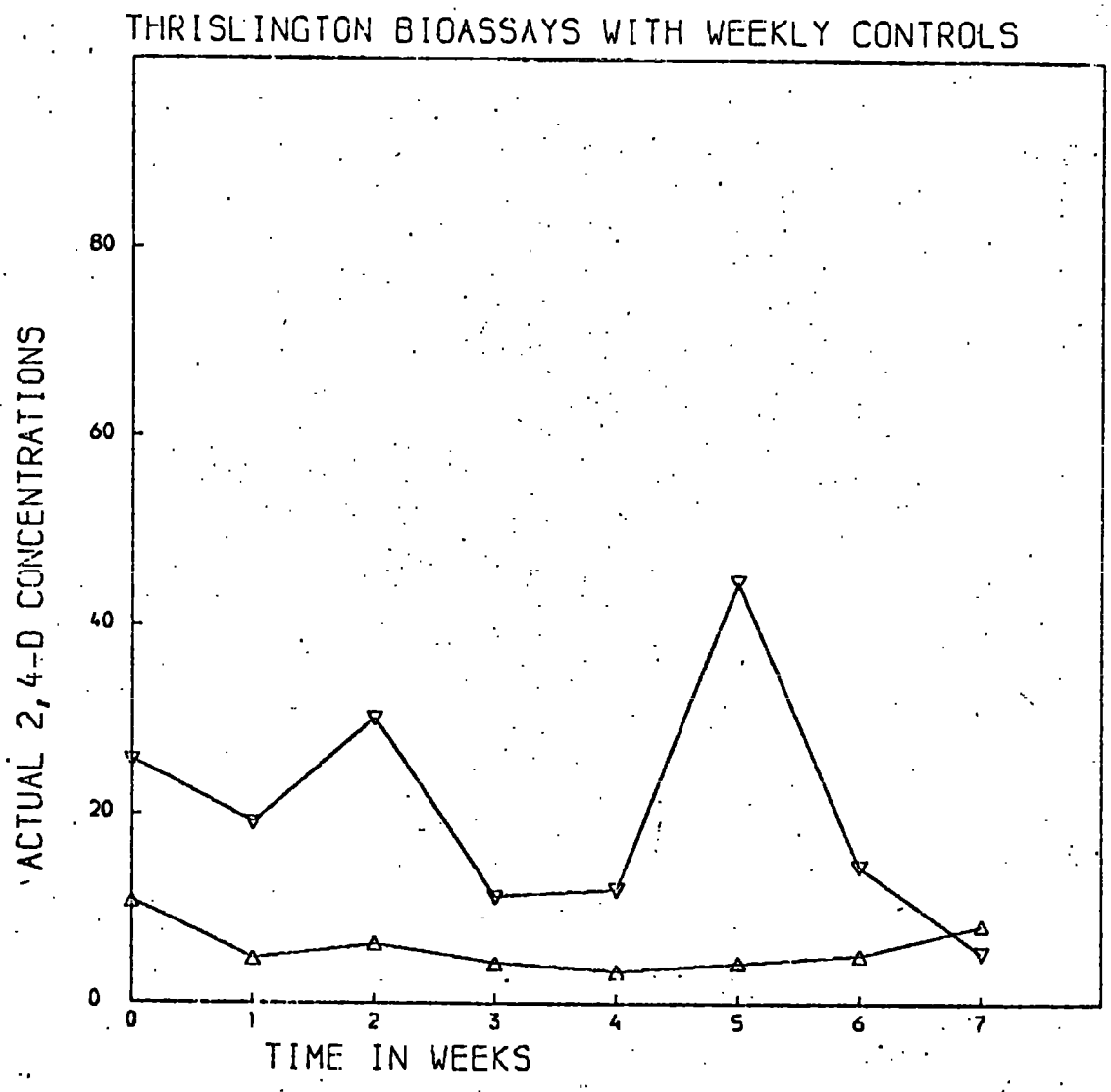

THRISLINGTON BIOASSAYS WITH WEEKLY CONTROLS
ACTUAL 2,4-D CONCENTRATIONS
0
20
40
60
80
0
1
2
3
4
5
6
7
TIME IN WEEKS

Figure 27: weekly bioassays using weekly controls for calculations - wood bottom, middle, top.

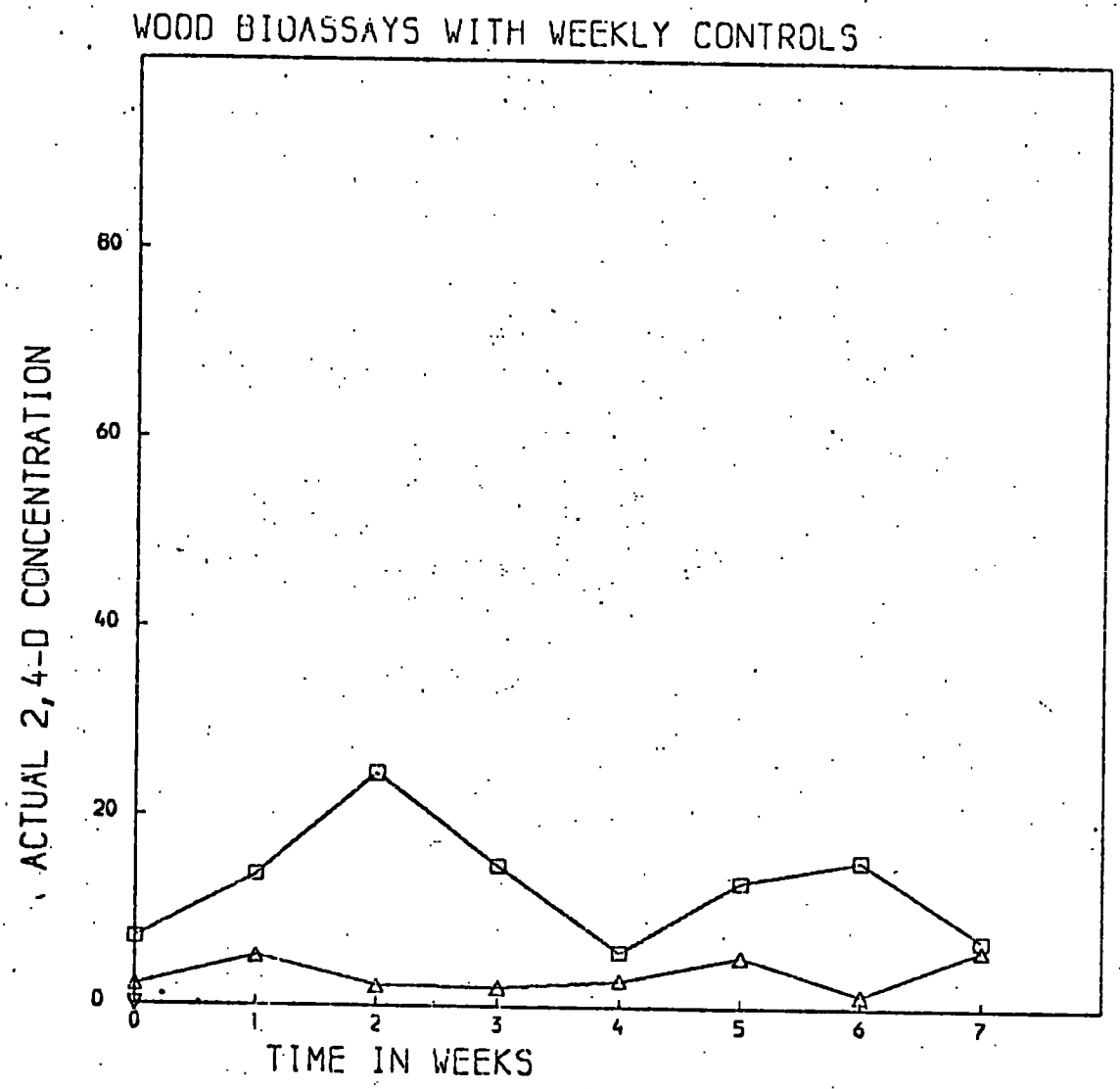

WOOD BIOASSAYS WITH WEEKLY CONTROLS
ACTUAL 2,4-D CONCENTRATION
80
60
40
20
0
0
1
2
3
4
5
6
7
TIME IN WEEKS

Figure 28: weekly bioassays using weekly controls for calculations - peat, bottom, middle, top.

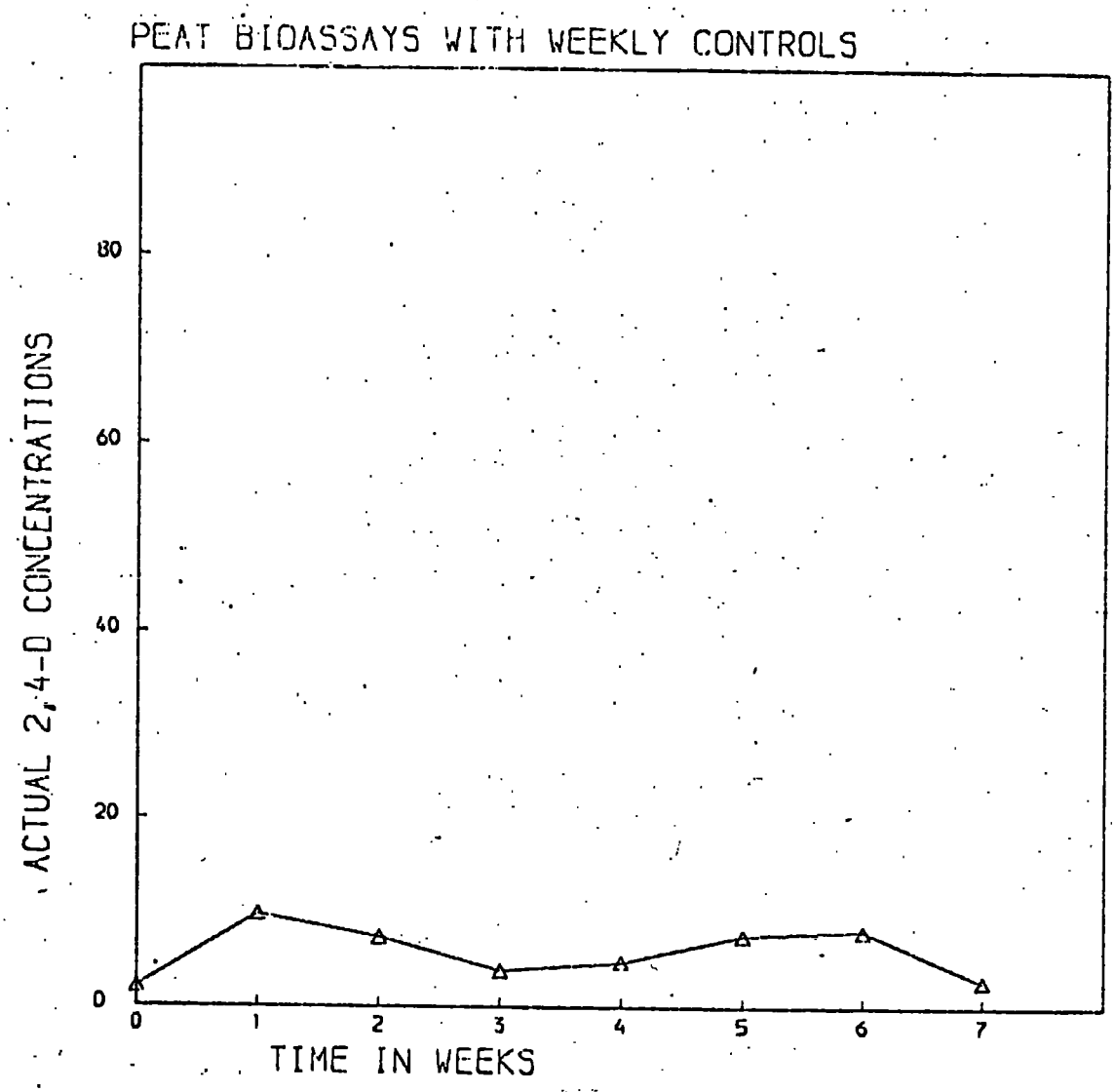

PEAT BIOASSAYS WITH WEEKLY CONTROLS
ACTUAL 2,4-D CONCENTRATIONS
80
60
40
20
0
0
1
2
3
4
5
6
7
TIME IN WEEKS

Figure 29: comparison of wet and dry soil from Thrislington Common: wet soil, using original controls for calculations - bottom, middle, top.

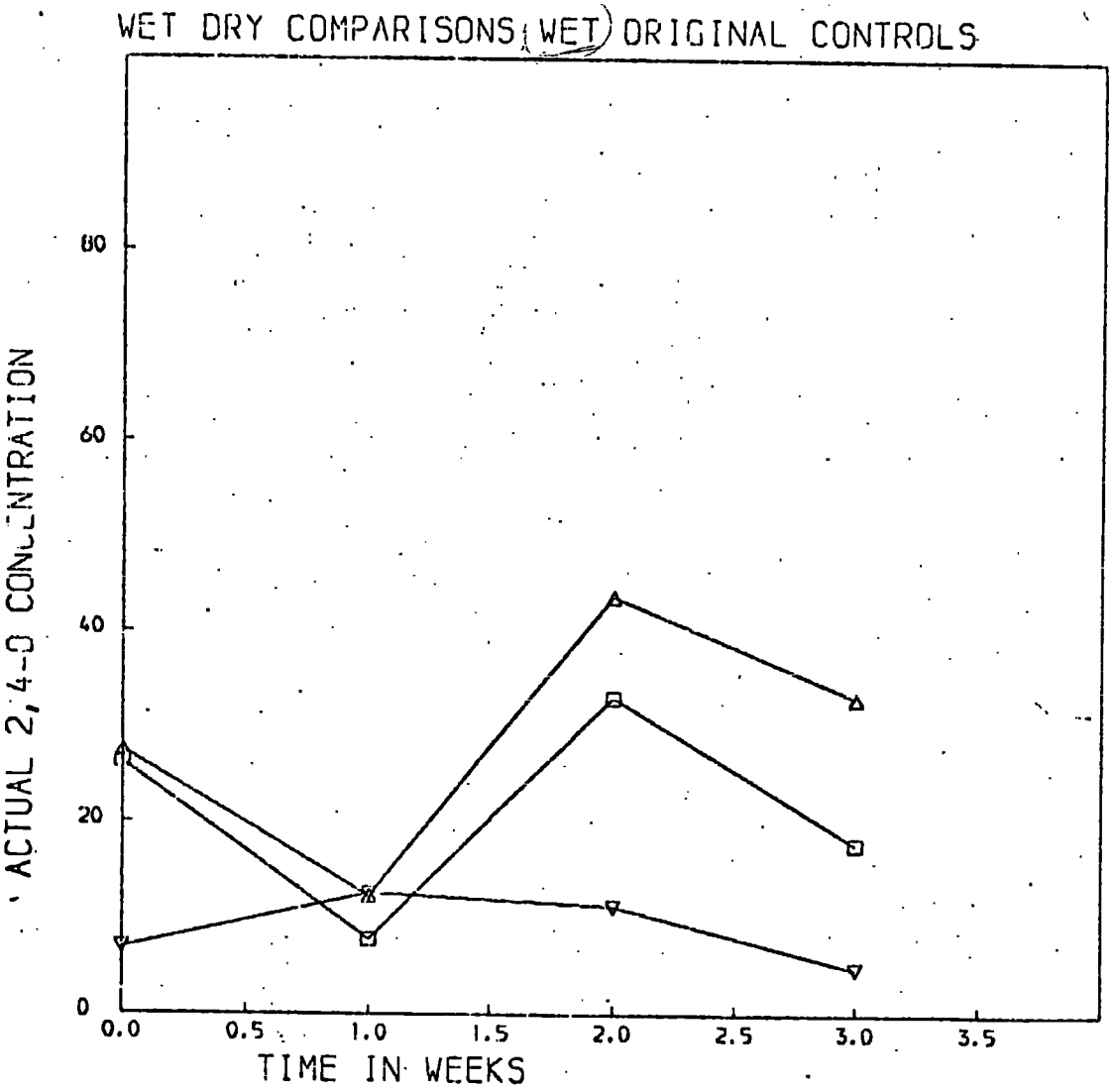

WET DRY COMPARISONS (WET) ORIGINAL CONTROLS
ACTUAL 2,4-D CONCENTRATION
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
TIME IN WEEKS

Figure 30: comparison of wet and dry soils from Thrislington Common: dry soil, using original controls for calculations - bottom, middle, top.

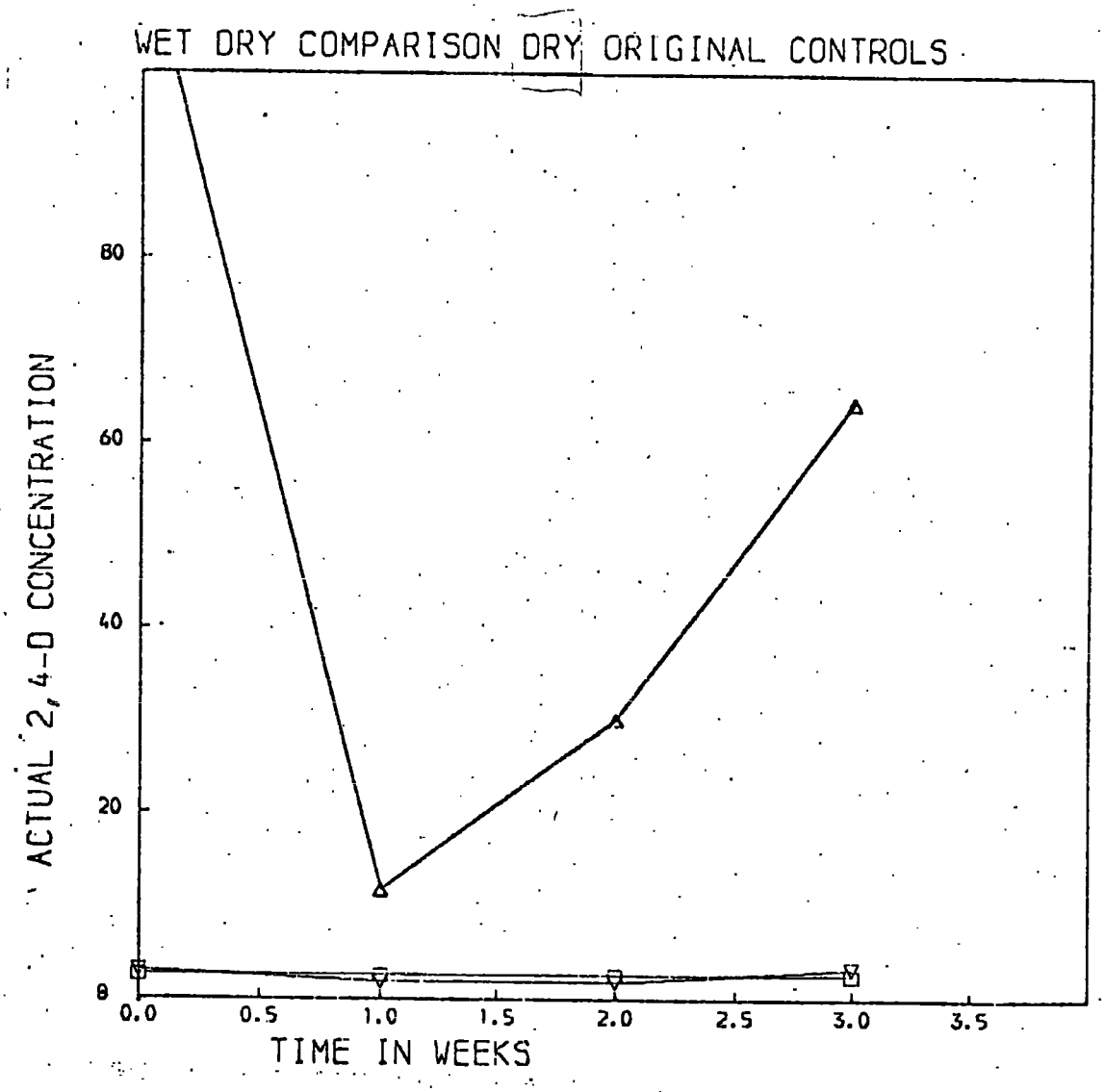

WET DRY COMPARISON DRY ORIGINAL CONTROLS
ACTUAL 2,4-D CONCENTRATION
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
TIME IN WEEKS

TABLE 13:

Thrislington Common field bioassays.

Results expressed as shoot indices where: shoot index is equal to the smaller of : I and $\frac{\text{growth of sprayed samples during bioassay}}{\text{mean growth of controls during bioassay}}$

See Appendix 3.

	Shoot index
I. Quadrats sprayed 31st May	
Bioassay set up 7th June	.43
Bioassay set up 15th June	1.0
II. Quadrats sprayed 27th June	
Bioassay set up 5th July	.55
Bioassay set up 12th July	1.0
Bioassay set up 20th July	.55
Bioassay set up 27th July	1.0
III. Quadrats sprayed 27th July	
Bioassay set up 3rd August	.67
Bioassay set up 10th August	1.0

TABLE 14:

Results

Bioassay results for 100ppm 2,4-D solution left in containers in greenhouse on 25th June.

	Week 0	Week 1	Week 2	Week 3
Mean growth of wheat shoot	.3	.13	.1	Completely evaporated
Standard deviation of mean	.14	.06	.0	

Figure 31: comparison of metabolic uptake of radioaction 2,4-D by wheat seeds on top soil from Thrislington Common and on top peat soil.

doesn't correlated with log d

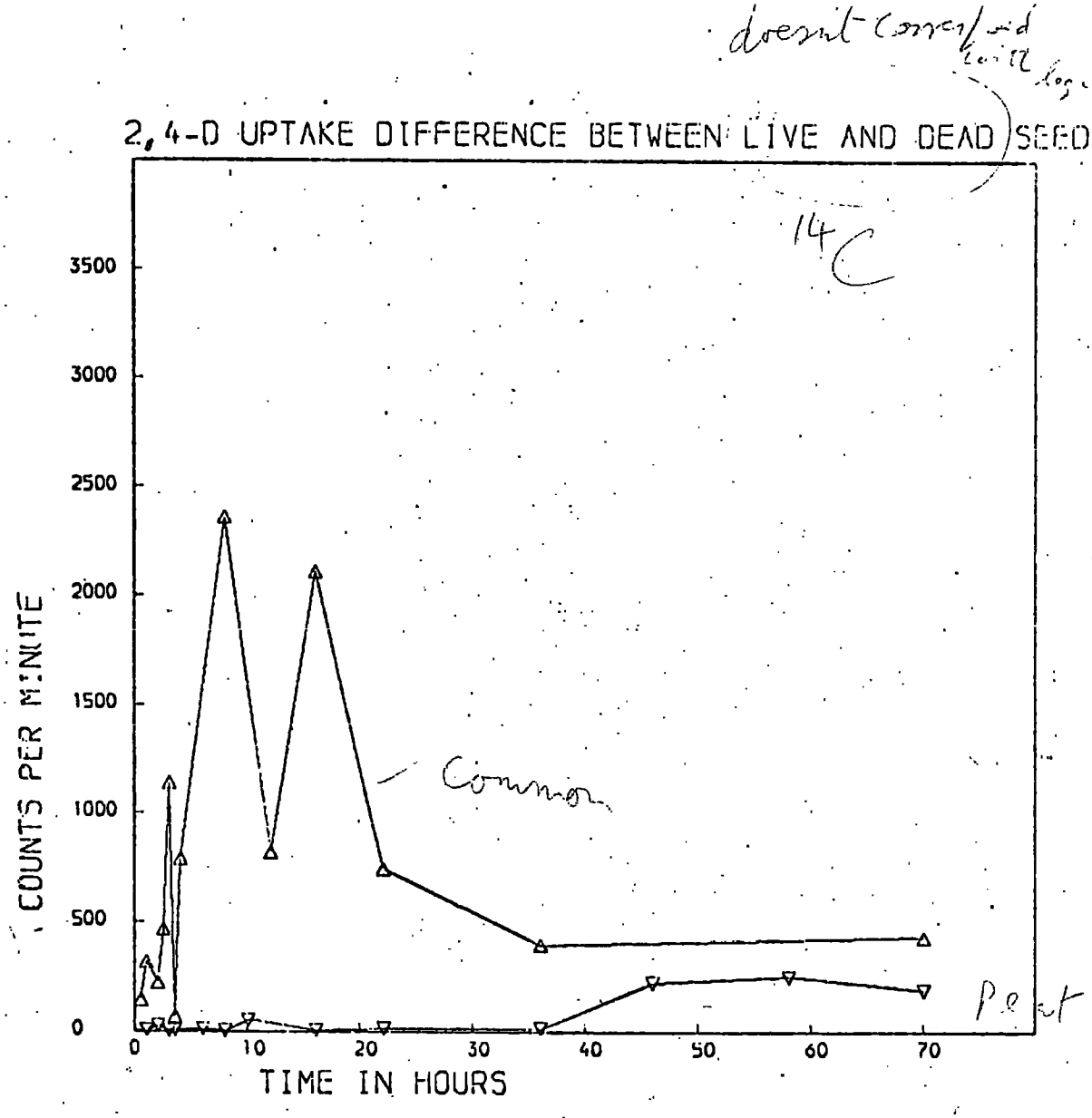


TABLE 15:

Radio-chemical comparison of soils before and after centrifuging with distilled water.

Sample	Soil Type	Treatment	counts per minute per gram of sample / counts per minute per gram of control expressed as percentage
A	Thrislington	Shaken & centrifuged with 10cm^3 water	.234
B	Peat	Shaken & centrifuged with 10cm^3 water	17.65
C	Thrislington	Shaken & centrifuged with 10cm^3 water	21.12
D	Peat	Shaken & centrifuged with 10cm^3 water	21.67
E	Thrislington	Shaken & centrifuged with 20cm^3 water	11.24
F	Thrislington	Shaken & centrifuged with 20cm^3 water	16.82

TABLE 16:

Radio-chemical comparison of seeds before and after shaking with distilled water.

	Before shaking	After 10 min. shaking	After 30 min. shaking
Counts/minute	3391.4	2893.8	1566.6
% of original amount of 2,4-D	100	85.3	46.2

CHAPTER 4 Discussion

The assessment of 2,4-D persistence and vertical movement in the soils from the five sites studied was carried out by using bioassays. As can be seen in Chapter 3, the results obtained from these were at best highly variable, and at worst meaningless. There was, by no means, a steady decline of 2,4-D in the soils, and much of the fluctuation is probably due more to error in the method than to changing concentrations in the soil. It seems probable that the bioassay method used to assess 2,4-D concentrations was unreliable because of failings both in the response of the wheat shoot to 2,4-D concentration, and in the conditions under which the bioassay was carried out. Ideally, much more time would have been given to the preliminary trials with different species and different possible measurements for the calculation of shoot indices.

The decision to use wheat seeds was based on their germination and growth on filter papers, not on the soils. It might have been better to use more than one species over the concentration range examined. It had been intended to assess concentrations of 2,4-D below 10 p.p.m. using cucumber seeds, but this had to be discarded part-way through the project as explained in the introduction.

The number of seeds sown and measured for each bioassay was limited by laboratory space available, and the feasability of measuring all the seeds within a few hours. More seeds would probably have reduced the variation in measurement, which would have been particularly desirable for the controls used to plot the graphs of shoot index against logarithm of concentration.

Ideally, too, bioassays would have been carried out more frequently, which would have made it easier to decide where there was a genuine decrease in 2,4-D, and to estimate the rate of degradation.

Both the germination and growth of seedlings, and the toxicity and persistence of 2,4-D are affected by environmental conditions such as temperature, light intensity, and soil moisture (Brown and Mitchell, 1948; Penfound and Minyard, 1947). Time did not allow the standardization of

watering techniques, neither of containers in the greenhouse, nor of soil in petri dishes for weekly bioassays, although it was attempted to water to field capacity. It was not possible to use a constant temperature and light growth chamber, owing to the number of petri dishes used in each bioassay.

It was hoped that by calculating shoot indices from weekly controls the effects of laboratory variations on seed growth would be partly cancelled. The soils from only three of the five sites, each at the three depths, were assessed in this way. The results from four out of these nine soil types could not be assessed because the control graphs for conversion of shoot indices to logarithm of concentration were not sufficiently close to a straight line. Only one of the remaining five soils showed a decline in 2,4-D concentration over the seven weeks. It seems highly improbable that this was because there was no degradation, since all the soils were kept fairly moist (De Rose, 1946; Ogle and Warren, 1954). In assessing persistence of 2,4-D, therefore, the results were obtained for shoot indices from one set of controls throughout.

Fluctuations in greenhouse conditions, including dry sunny spells where temperatures reached 50°C, meant that soil dried differently between bioassays. This was rectified to some extent by watering mid-week when very dry. These high temperatures might be expected to lead to faster breakdown than under field conditions.

One other intrinsic problem with bioassays is the delay - in this case of three days - between sampling the soil and starting the bioassay and its completion.

Much research on 2,4-D since the late fifties or early sixties has used radioactive tracers (Yamaguchi and Crafts, 1958), the 2,4-D labelled in the carboxyl group, or occasionally in the ring, with ^{14}C. This eliminates most of the problems and inaccuracies associated with bioassays, and is faster. It is unsuitable, though, for much work with soil

degradation, because it is not possible, by this method, to distinguish between 2,4-D and its breakdown products while these remain in the soil. It is better suited to studies of absorption and translocation. Gas chromatography has been used frequently for studies of breakdown (Hance and McKone in Audus, 1976), but it was not possible to set it up on such a short time scale.

The weekly concentration of 2,4-D, as obtained using the original controls throughout to calculate shoot index, shows a decline in most of the soils, but, in general, not a very consistent one. Neither the decline in logarithmic nor in actual concentration showed any significant straight line relationship (Table 33), nor was there any other obvious relationship. It was therefore not possible to calculate the rate of decline. In none of the soils did 2,4-D fall to, and remain at, 0 p.p.m. The length of time that 2,4-D remained in the soil was taken to be the number of weeks before the concentration fell, and afterwards remained, below 20 p.p.m. This is, perhaps, a somewhat arbitrary estimation of persistence, but seemed, from examination of the graphs, to be the most appropriate.

The soils were compared on this basis, and correlation sought between persistence of 2,4-D in each soil, and physical and chemical properties of the soils. Soil properties have previously been found to affect persistence and toxicity of 2,4-D in the soil. High pH on to liming soil is often thought to increase its persistence and toxicity, but has not always been found to do so (Hanks, 1947). Organic matter may affect toxicity by making 2,4-D unavailable, or by speeding up degradation by increasing the number of microorganisms present. Upchurch and Mason (1962) found some correlation between effective toxicity, as measured by 50% growth response in bioassays, and certain soil properties. They obtained correlation coefficients, r, greater than, or equal to, 0.85 for exchangeable calcium (r= 1), total exchangeable bases (r = 0.99), cation exchange capacity (r = 0.95), free drainage value (r = 0.87) and pH (r = 0.85). They found no correlation

($|r| < 0.25$) between particle size of exchangeable magnesium and 50% growth response.

No significant correlations between persistence of 2,4-D and soil properties of the soils studied here were found. The only correlation coefficient with a value of $|r| > 0.5$ was obtained from the linear regression of cation exchange capacity on soil persistence ($r = 0.58$).

The results for total exchangeable bases were not considered, as they seemed too unlikely. Soils of similar type, such as those from adjoining depths at the same site, showed dissimilar results, and the results did not correspond very well with cation exchange capacity or with exchangeable calcium and magnesium. There was possibly slight loss due to spattering in heating the leachates to dryness, and the high temperature of ignition (500°C) recommended in Metson's method may alter the exchangeable bases in the soil. However, since the exchangeable calcium and magnesium results obtained from the final titre were fairly consistently about twice those obtained from the leachate (in p.p.m.) it seems likely that the errors were often caused by very small differences in the titration making large differences in the final result.

Leaching is also dependent on soil type, being greater in sandy than in clay soils or humus. Ogle and Warren (1954) found that sixteen inches of water were required to free all toxicity from a highly organic soil, and Audus (1952) also found that the 2,4-D leached from soils was inversely related to the amount of organic matter in the soil.

In the five sites studied, 2,4-D appeared to be held for the longest time in the top soils of Thrislington Common and the wood soil. Although the top soil of Thrislington Common was relatively high in organic matter, the top soil of the wood was lower in organic matter than the top soil of the turf. Nor does it seem likely that it was held for longer by the roots of growing vegetaion, since there was more vegetation in the turf. Also, in comparing wet and dry soils from Thrislington Common, the bottom soil of both wet and dry samples had higher concentrations than either the middle or top soils.

In the dry soil, no 2,4-D appeared to be retained by the top or middle soils; in the wet soil, the middle had little or no 2,4-D and showed little variation, whereas the top followed a similar pattern to the bottom, but with slightly lower concentrations. It thus appears that there may be more chance of 2,4-D being retained on wet soil, and that it may leach straight through a well-drained dry soil. This might also have caused discrepancies between unevenly watered containers.

The bottom soils, especially Thrislington Common, show a fairly consistent dip in 2,4-D concentration at the end of the first week. This may be due to the herbicide being absorbed onto soil colloids during the first few days, and later released. This might mean it was unavailable to the wheat seeds at the end of the first week, but was taken up by them again a week later.

It was not really possible to look at leaching on the peat soil as there was little relationship between wheat growth on the peat top and middle soil and 2,4-D concentration although it did seem to persist a long time in the bottom soil. The linear regression of shoot index on logarithm of 2,4-D concentration was not significant in either case, and there seemed little, if any, relationship between them. Wheat seeds grew considerably less well on the unsprayed peat than on the other soils, but at high 2,4-D concentrations on the top and middle peat soil, often grew much better than on other soils. This suggested either that the 2,4-D was immediately locked up in the peat, or that it was immediately degraded. The latter explanation seemed less likely, since relatively few microorganisms are able to live in so acid a soil. Top soil from the peat, and from Thrislington Common, were sown with wheat seeds immediately after adding radioactive 2,4-D, in order to observe uptake by the seeds and see if any was being taken up on the peat. As can be seen from the graphs, there is a very significant difference in uptake. Very little 2,4-D enters the seeds on the peat either metabolically or physically, though there is a slight but steady rise in uptake over the three days studied, possibly suggesting a gradual release. The uptake by seeds on the magnesium

limestone was more erratic, but was an order of magnitude higher, and showed a peak about twenty-four hours after the experiment started. This certainly corroborated the evidence from bioassays that the growth of seeds on the top peat soil was unaffected by 2,4-D concentration, but did not explain why. The counts per minute per gram of soil of Thrislington Common was about seven times higher than that of the peat at the end of the three days, which suggests that the 2,4-D had been broken down and the carbon - 14 released as carbon dioxide, rather than locked up in the soil. However, it is felt that this is insufficient evidence to base any conclusions on.

Comparing the counts per minute per gram of soil after shaking with water and centifruging with soil that had not been treated with water, it appears that most of the radioactivity (up to 90% at least) can be washed out. Slightly more was washed out using $20cm^3$ water than using $10cm^3$, but there was no marked difference between the peat and Thrislington Common. This seems surprising in view of the results with seed uptake, though the soils were compared after the study of seed uptake (i.e. four days after the 2,4-D was put on the soil) by which time the seeds on the peat were starting to take up more, whereas active uptake by seeds on Thrislington Common soil had declined. It may be that 2,4-D would have been equally available to seeds on both soils by this stage. Alternatively, it may be that of the 2,4-D in the soil, proportionally as much could be washed out of, or taken up from, the peat, but there was less in the peat soils due to rapid breakdown. This, though, does not explain the release effect.

At least 50% of 2,4-D could be washed out of germinated seeds by shaking with water. This suggested that at least a large percentage of the 2,4-D had not been metabolized within the cells. Previous work with barley roots showed that all the 2,4-D taken up could be leached out with prolonged washing. The field experiments all indicate that there was no measurable 2,4-D after two weeks, but, in the only month where four weeks of results are available, the apparent concentration was up again in the third week, and back to zero again in the fourth.

It is impossible to say on so little evidence whether this was indeed due to the 2,4-D, and there is some locking up after two weeks or whether the 2,4-D has really disappeared from the top 4cm. at the end of two weeks. The latter would approximately correspond to greenhouse results, since the soil taken from the field was a mixture of the top and part of the middle layers used in the greenhouse.

When 2,4-D was left in solution in the greenhouse for two weeks, there was no sign of degradation. The soil apparently increased in concentration until it evaporated.

2,4-D did not appear to affect nitrogen content, at least not in the short term studied. Although there were consistent differences between bottom, middle and top soil in both sprayed and unsprayed samples, there was basically no difference between sprayed and unsprayed samples at one depth. There appeared to be a slight increase in nitrogen content of the middle sprayed soil, but it was not significant.

APPENDICES

APPENDIX 1: Soil properties.

TABLE 17:

Field Capacity

Soil Type	Thrislington Common		Turf		Wood			Loam		Peat	
Weight of wet soil in g.	227.05	221.62	227.88	180.60	245.94	239.38	239.58	216.89	226.90	187.01	210.79
Weight of dry soil in g.	134.21	130.83	133.45	105.88	154.36	150.59	152.42	144.77	151.33	52.66	59.46
Weight of water contained in soil in g.	92.84	90.79	94.43	74.72	91.58	88.09	87.16	72.12	75.57	134.35	150.7
% of water held = weight of water x 100 weight of dry soil	69.2	69.4	70.8	70.6	59.3	58.5	57.2	47.7	49.9	255.1	4.5

TABLE 18:

Organic Carbon (Walkley-Black method):

Soil Tyoe	Weight of oven-dry soil in grams	Ammonium ferrous oxide titre in cm^3
Thrislington Common Bottom	.68768	6.9
Thrislington Common Middle	.70650	5.1
Thrislington Common Top	.66523	.9
Turf - Bottom	.64036	10.3
Turf - Middle	.56920	6.6
Turf - Top	.54440	4.8
Wood - Bottom	.59265	10.3
Wood - Middle	.48535	12.1
Wood - Top	.55233	7.7
Loam - Bottom	.64484	10.3
Loam - Middle	.63005	10.7
Loam - Top	.57262	10.1
Blank		20.4 (Average of two determinations)

Calculation:

$$\% \text{ oxidizable organic carbon (uncorrected} = \frac{(\text{blank titre} - \text{actual titre}) \times .3 \times M}{\text{weight of oven-dry soil in g.}}$$

where M = concentration of ammonium ferrous sulphate (approximately :5M)

TABLE 19:

Organic Carbon

Loss on ignition using Ball's regression (Ball, 1964).

Soil Type	Weight of oven-dry (110°C) soiling	Weight of soil after ignition in g.	Matter lost during ignition in g.	Loss on ignition %	Organic carbon (from Ball's regression) %
Peat Bottom	17.0	14.03	2.97	17.47	7.50
Peat Middle	13.8	6.26	7.54	54.64	24.63
Peat Top	6.81	1.12	5.69	83.55	37.87

Ball's regression: $y = 0.458x - 0.4$

where x is the percentage loss on ignition and

y the percentage organic carbon.

TABLE 20:

Soil Particle Analysis:

Soil type	Initial wt. of soil	Wt.of soil after pre-treatment W_B	Wt.of soil on coarsest sieve 2.4-6mm W_C	Wt. of soil on medium sieve .6 - .21mm W_M	Wt. of soil on finest sieve .21 - .075 W_F	Wt. of soil from 1st pipetting W_1	Wt. of soil from 2nd pipetting W_2	Wt. of soil from 3rd pipetting W_3
TCB	17.979	16.34	2.58	4.33	2.57	.045	.028	.028
TCM	17.674	16.99	.99	4.61	3.36	.053	.040	.031
TCT		----------	----------	No Results	----------	----------	----------	----------
TB	14.230	12.88	.44	1.17	2.18	.090	.074	.070
TM	14.368	13.27	.74	1.38	2.3	.083	.074	.066
TT	12.960	11.72	.22	3.92	.03	.072	.059	.053
WB	20.886	17.81	.70	1.51	3.08	.150	.106	.081
WM	17.890	14.98	.61	1.13	2.61	.093	.073	.065
WT	17.137	14.62	.33	3.86	0.0	.105	.084	.069
LB	25.138	23.16	1.44	3.05	5.70	.119	.087	.071
LM	19.715	18.86	1.25	3.84	5.96	.072	.061	.043
LT	21.405	20.62	1.02	3.53	6.36	.079	.067	.048

Soil Particle Analysis

Calculations

Fine sieving

Percentage of number 25 (2.4-0.6mm) sieve $= \frac{100W_c}{W_B}$

Percentage on number 72 (0.6-0.21mm) sieve $= \frac{100W_M}{W_B}$

Percentage on number 200 (0.21-0.075mm) sieve $= \frac{100W_F}{W_B}$

Sedimentation

Weight of solid material in $500cm^3$ of suspension, M_i is given by:

$$M_i = \frac{W_i}{V_p} \times 500 \text{ g}$$

where i = 1, 2 or 3 represents the 1st, 2nd or 3rd pipetting respectively, and where V_p is the volume of the pipette

$$V_p = 9.9125 \quad \text{(previously calculated).}$$

Percentage of medium silt (.02 - .006mm) $= \frac{M_2 - M_3}{W_b} \times 100$

Percentage of clay (less than .002mm) $= \frac{M_3 - M_4}{W_b} \times 100$

Where M_4 is the weight of sodium hexametaphosphate in $500cm^3$, calculated from a blank sample without soil.

The percentage of coarse silt was obtained from the other results by subtraction.

TABLE 21:

Cation Exchange Capacity

Soil Type	Weight of 1g of soil + tube + seal in g W_1	Weight of soil + tube + seal after discarding distilled water washing in g W_2	Actual Titres in cm^3	Average Titre used in calculation in cm^3 T_1
Thrislington Common Bottom	13.44	14.49	7.7 7.9	7.8
Thrislington Common Middle	19.04	20.24	9.0 9.0	9.0
Thrislington Common Top	13.54	14.74	8.5 8.6	8.55
Turf Bottom	13.30	14.17	9.2	9.2
Turf Middle	41.96	42.87	9.6	9.6
Turf Top	42.26	43.42	9.0 9.0	9.0
Wood Bottom	13.53	14.33	9.7 9.8	9.75
Wood Middle	13.67	14.54	9.7	9.7
Wood Top	13.30	14.83	9.4	9.4
Loam Bottom	19.07	20.06	9.9	9.9
Loam Middle	13.34	14.29	9.8	9.8
Loam Top	13.50	14.48	11.5 11.5	11.5
Peat Bottom	18.94	19.79	8.1 8.2	8.15
Peat Middle	18.97	21.73	4.4 4.4	4.4
Peat Top	19.04	24.25	8.3 8.1 8.3	8.23

Calculation

C.E.C. = $8(T_B - T_2)$ me/100g soil

where $T_2 = T_1 (100 + W_2 - W_1)/100$ cm^3

and T_B is the titre of the blank titration.

TABLE 22:

Total Exchangeable Bases (Appendix)

Soil Type	Titre of .1M Ammonium hydroxide in cm^3
Thrislington Common Bottom	8.7
" " Middle	9.0
" " Top	9.1
Turf Bottom	9.75
" Middle	9.85
" Top	8.7
Wood Bottom	9.3
" Middle	9.05
" Top	9.25
Loam Bottom	9.75
" Middle	9.75
" Top	9.7
Peat Bottom	9.3
" Middle	9.85
" Top	9.75
Control	9.8

Calculation

Total Exchangeable Bases (T.E.B.) in m.e./100g soil = $\frac{10mV}{wr}$

where m is the number of cm^3 of .1M HCL used to neutralize the residue corresponding to 10g. soil; Vcm^3 is the total volume of leachate; r is the aliquot evaporated; W_g is the weight of soil leached. W = 5, V= 250, = 25.

TABLE 23:

Exchangeable Calcium and Magnesium

Soil Type	Calcium		Magnesium	
	Results from leachate (see methods) ppm	Results from titrated solution (see methods) ppm	Results from leachate ppm	Results from titrated solution ppm
Thrislington Common Bottom	83	170	22.1	42
Thrislington Common Middle	86	170	17.8	32
Thrislington Common Top	97	185	19.8	39
Turf Bottom	55	98	6.5	10.2
Turf Middle	58	103	6.7	12.0
Turf Top	65	120	8.2	14.7
Wood Bottom	47	106	5.4	14.5
Wood Middle	48	98	5.8	11.1
Wood Top	56	98	7.1	12.1
Loam Bottom	69	129	3.6	6.3
Loam Middle	63	112	3.4	5.8
Loam Top	64	118	3.7	6.5
Peat Bottom	6	13	1.6	2.8
Peat Middle	10	18	2.8	4.9
Peat Top	28	51	6.4	11.4
Control	1	6	0.9	1.4

TABLE 24:

Nitrogen determinations

Soil Treatment	Soil depth	Titre of .02M hydrochloric acid in cm^3
Control I	Bottom	2.8
	Middle	2.9
	Top	3.0
Control II	Bottom	1.9
	Middle	2.2
	Top	2.9
Control III	Bottom	2.0
	Middle	-
	Top	3.6
Sprayed with 2,4-D 3 days before nitrogen analysis I	Bottom	2.1
	Middle	2.6
	Top	2.7
Sprayed with 2,4-D 3 days before analysis II	Bottom	2.3
	Middle	2.8
	Top	3.4
Sprayed with 2,4-D 10 days before analysis	Bottom	2.0
	Middle	3.0
	Top	3.1
Sprayed with 2,4-D 11 days before analysis	Bottom	2.45
	Middle	2.75
	Top	3.1

Nitrogen determinations (Appendix)

Calculation

Let a cm^3 be the titre of .02 HCL used; and Wg. the weight of soil.

Then:

$$\% \text{ nitrogen in sample} = \quad x \ \frac{.28}{1000} \ x \ \frac{250}{10} \ x \ \frac{100}{W} = \quad x \ .14$$

where $\frac{.28}{1000}$ is the conversion of mg of nitrogen to g of nitrogen, and $\frac{250}{10}$ is the dilution factor.

1. Chemical Reagents Used in Soil Analyses:

Oxidizable organic carbon:

Potassium dichromatic solution .17M : 49.04g pure potassium dichromatic dissolved in water, and the solution diluted to 1L.

Ammonium ferrous sulphate solution .5M : 196g ammonium ferrous sulphate dissolved in water, $5cm^3$ concentrated sulphuric acid added, and the solution made up to 1L with distilled water.

Particle Analysis:

Sodium hexametaphosphate solution. 33g. sodium hexametaphosphate and 7g. sodium carbonate dissolved in distilled water and made up to 1L.

Total Exchangeable Bases:

Ammonium acetate solution, neutral, 1.0M : $57cm^3$ glacial acetic acid and $68cm^3$ concentrated ammonium hydroxide added to $800cm^3$ distilled water and diluted to 1L. The solution was adjusted to pH7.0.

Cation Exchange Capacity:

Triethanolamine solution: $90cm^3$ triethanolamine were diluted to 1L and adjusted to pH8.1 using 2M hydrochloric acid. The solution was then diluted to 2L.

Buffered barium chloride solution: 244g. barium chloride was dissolved in 1L water, and this was mixed with 1L triethanolamine solution.

EDTA solution: 3.723g of the disodium salt were added to 1L of water.

Catechol violet indicator: .1g was added to $100cm^3$ water.

2. Magnesium sulphate solution: 6.2g in 1L.

Total Nitrogen:

Boric acid +mixed indicator : 40g boric acid dissolved in $800cm^3$ hot distilled water. $20cm^3$ mixed indicator (.5g bromocresal green and .1g methyl red in $100cm^3$ ethanol, with pH then adjusted to 4.5) was added, and the solution diluted to 1L.

Radiochemical Experiments:

Scintillation fluid: two parts toluene to one part triton x - 100, with diphenyl oxazole (PPO) added at the rate of $4g./dm^3$ toluene.

APPENDIX 2: Preliminary Experiments for bioassays.

TABLE 25:

Preliminary Experiments for Bioassays

Results of initial bioassay tests of the growth of cress and wheat seeds on filter paper at different concentrations of 2,4-D (see 6.1.1. and 6.1.2. in 'Methods').

Concentration of 2,4-D in ppm	0.0	1.0	2.5	5.0	7.5	10.0	25.0	50.0	75.0	100.0
WHEAT SHOOT length in cm.	4.1	3.4	3.5	3.5	3.2	2.8	2.0	2.3	1.7	1.3
	3.7	3.5	3.8	2.2	2.8	1.5	1.5	1.6	1.4	.9
	3.9	3.0	3.7	2.3	2.1	1.9	1.5	1.1	1.2	.5
	1.0	1.8	3.0	2.5	2.7	1.3	1.5	.4	1.1	-
	-	-	1.4	0.0	.7	.5	.1	.3	-	-
Mean of length in cm	3.2	2.9	3.1	2.6	2.3	1.6	1.3	1.1	1.4	.9
Standard deviation	1.5	0.8	1.0	0.6	1.0	.8	.7	.8	.3	.4
WHEAT ROOT length in cm.	4.9	.9	.9	.9	1.1	.3	.3	.6	.1	.2
	2.7	.8	1.6	1.0	.2	.3	.5	.6	.3	.4
	4.0	.7	.9	.5	1.5	.4	.2	.4	.2	.1
	.4	1.0	.8	.6	.4	.3	.1	.1	.5	-
	-	-	.5	-	.1	.1	-	.1	-	-
Mean of length in cm	3.0	.9	.9	.8	.7	.3	.3	.4	.3	.2
Standard deviation	2.0	.1	.4	.2	.6	.1	.2	.3	.2	.2
WHEAT SHOOT + ROOT Mean of length in cm.	6.2	3.8	4.0	3.4	3.0	1.9	1.6	1.5	1.7	1.1
CRESS SHOOT length in cm.	2.0	.8	.7	.2	.2	.2	.1	.1	.1	.1
	1.7	.6	.6	.2	.2	.2	.1	.1	.1	.1
	1.8	.5	.9	.2	.3	.3	.2	.2	.1	.1
	1.4	.6	.4	.2	.4	.3	.4	.2	.2	.2
	1.8	.7	.5	.2	.4	.3	.2	.2	.2	.2
Mean of length in cm.	1.7	.6	.6	.2	.3	.3	.2	.2	.1	.1
Standard deviation	.2	.1	.2	.0	.1	.05	.1	.05	.05	.05
CRESS ROOT length in cm.	6.5	.2	.3	.2	.2	.2	.2	.1	.1	.1
	7.7	.2	.3	.2	.2	.2	.2	.1	.1	.1
	8.7	.3	.4	.2	.2	.2	.2	.1	.1	.1
	4.1	.2	.3	.2	.2	.2	.3	.2	.2	.1
	7.2	.2	.1	.2	.2	.2	.3	.2	.2	.2
Mean of length in cm.	6.8	.2	.3	.2	.2	.2	.2	.1	.1	.1
Standard deviation	1.7	.04	.1	0	0	0	.05	.05	.05	.04

TABLE 26:

Preliminary Experiments for Bioassay.

To look at variation in shoot growth of previously germinated wheat seeds. Growth in cm. over 48 hours of previously germinated wheat seeds in different concentrations of 2,4-D.

Concentration of 2,4-D in p.p.m.	Thrislington Common			Turf			Wood			Loam			Peat		
	Bot.	Mid.	Top	Bot.	Mid.	Top	Bot.	Mid.	Top	Bot.	Mid.	Top	Bot.	Mid.	Top
100	.9	1.1	.3	.2	1.6	1.4	.1	.4	.1	.4	.2	.6	.2	.6	.4
	1.8	3.7	1.8	1.9	1.6	1.4	1.9	2.1	1.8	1.3	2.2	1.9	1.2	1.6	1.4
	1.5	1.2	1.7	3.6	1.4	1.5	1.6	2.2	1.5	3.4	1.0	1.6	2.1	2.0	1.8
	.6	1.8	1.1	.8	1.6	1.2	.3	1.1	.2	.2	1.0	1.9	1.2	1.7	.8
Mean	1.2	2.0	1.2	1.6	1.6	1.1	1.0	1.5	.9	1.3	1.1	1.5	1.2	1.5	1.2
standard deviation	.5	1.2	.7	1.4	.1	.4	.9	.9	.9	1.5	.8	.6	.8	.6	.6
50	1.1	0.0	.4	.5	.9	.1	.1	.6	.5	.9	.7	2.5	.2	1.0	.7
	.5	1.0	1.0	.8	.2	.6	.9	1.7	1.4	1.3	1.8	0.0	1.1	1.8	1.0
	1.5	2.0	.8	2.4	1.5	0.0	2.4	1.1	2.1	1.8	1.1	.8	1.3	2.0	1.7
	1.4	1.8	2.1	1.2	1.1	0.0	1.4	1.4	1.8	-	1.4	.6	1.5	2.2	1.8
Mean	1.1	1.2	1.1	1.2	.9	.2	1.2	1.2	1.5	1.3	1.3	1.0	1.0	1.8	1.3
standard deviation	.5	.9	.7	.8	.5	.3	1.0	.5	.7	.5	.5	1.1	.6	.5	.5
10	1.4	1.6	.9	.9	1.4	1.5	1.5	.8	.3	1.8	.8	1.4	.9	1.3	1.5
	2.0	.6	1.3	.1	.9	.7	.3	.8	.4	1.7	1.2	.5	.4	1.1	1.2
	1.2	1.4	2.4	2.3	1.6	1.5	2.0	2.2	1.8	.6	2.5	2.6	1.6	2.1	1.7
	2.3	1.8	.8	1.9	1.4	-	1.8	1.5	2.6	1.1	2.2	1.4	2.4	1.5	2.6
Mean	1.7	1.4	1.4	1.3	1.3	1.2	1.4	1.3	1.3	1.3	1.3	1.5	1.3	1.5	1.8
standard deviation	.5	.5	.7	1.0	.3	.5	.8	.7	1.1	.6	.8	.9	.9	.4	.6

TABLE 27:

Raw data for controls used for plotting graphs of shoot index against logarithm of 2,4-D concentration.

	Thrislington Common			Turf			Wood			Loam			Peat		
	TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
Shoot length in cm.	3.0	2.3	1.4	2.1	2.1	1.8	2.2	2.2	2.9	2.2	1.5	2.0	1.6	0.5	0.7
	1.7	1.3	0.2	2.4	1.4	1.5	2.3	2.2	2.1	1.8	1.4	1.9	1.5	0.6	0.4
	1.0	0.3	0.1	0.6	1.2	1.4	1.7	1.5	1.6	1.6	0.3	1.8	1.0	0.8	0.3
	0.8	0.1	-	0.5	-	0.9	1.2	0.9	0.3	1.2	0.4	1.0	0.3	0.7	0.5
	-	0.1	-	-	-	0.4	-	0.9	0.2	0.2	-	0.3	-	0.4	0.3
Mean of shoot length	1.63	0.82	0.57	1.4	1.57	1.2	1.85	1.54	1.42	1.4	0.9	1.4	1.1	0.6	0.44
Standard deviation	0.99	0.97	0.72	0.99	0.47	0.55	0.51	0.65	1.16	0.76	0.64	0.73	0.59	0.16	0.17

TABLE 28:

Values for shoot indices used for plotting control graphs of shoot indices against logarithm of 2,4-D concentration.

2,4-D concentration in p.p.m.	TCB	TCM	TCT	TB	TM	TT	WB	WM	WT
100	0.24	0.46	0.53	0.09	0.08	0.24	0.14	0.16	0.17
50	0.39	0.32	0.91	0.44	0.44	0.23	0.12	0.29	0.29
10	0.55	1.00	1.00	0.82	0.45	0.53	0.49	0.31	0.74
0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

2,4-D concentration in p.p.m.	LB	LM	LT	PB	PM	PT
100	0.11	0.31	0.15	0.54	0.90	0.77
50	0.21	0.56	0.22	0.49	1.00	0.84
10	0.47	0.73	0.45	0.75	0.98	1.00
0	1.00	1.00	1.00	1.00	1.00	1.00

TABLE 29:

Shoot length of wheat in cm.

First set of weekly bioassays - raw data.

Week 0

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
.2	.6	1.1	1.2	1.1	1.2	1.0	.9	.8	1.0	1.1	.7	1.4	.7	.5
.6	1.2	1.1	.8	1.1	1.2	.9	.8	.4	.6	.8	.5	.9	.5	.2
.2	1.0	.5	.3	.8	.8	1.5	.9	-	.7	.7	.4	.7	.4	.2
.2	.7	-	.2	.7	-	1.0	.5	.1	1.1	.6	.1	.2	.5	.2
.1	.1	-	.1	-	.1	-	.2	.1	.6	.4	.1	.3	.5	.1
.4	.9	1.4	.5	.6	1.3	1.7	.7	.8	.7	.4	.5	.8	.2	.4
.4	.7	1.1	.7	.7	1.3	1.5	.3	.2	.8	.3	.5	1.2	.7	.6
.3	.7	1.2	.3	.2	1.2	1.4	.2	.3	.5	.5	.3	.9	.5	.2
.5	.9	.6	.6	.4	.6	1.0	.2	.1	.1	.2	.3	.5	.3	.2
-	1.1	.2	.1	-	.2	.2	-	.2	.0	-	.1	.2	.6	-
.5	1.0	.7	.5	.6	.6	1.4	.2	.9	.9	1.0	.3	1.0	.3	.4
.7	.8	.6	.6	1.0	.8	1.4	.3	.6	1.0	1.0	.2	1.3	.6	.1
.9	.7	.2	.7	.6	.4	1.0	.2	.5	.7	.8	.2	.7	.2	.2
.5	.7	.7	.2	.2	.2	1.0	.1	.2	.5	.8	.1	.5	.5	.2
.9	.5	.1	.3	.1	.2	.4	.2	.1	.5	.2	.1	.5	.5	.3

Week 1

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
2.3	1.5	1.9	1.5	1.1	1.1	1.5	1.4	.4	1.4	1.3	1.0	.7	1.0	.9
2.2	2.4	.8	1.6	1.3	1.8	1.6	1.6	.8	1.6	1.0	.6	1.0	.9	1.2
2.3	2.1	1.5	.8	1.2	.1	1.4	1.1	.5	1.7	.6	.5	1.3	.3	1.1
1.6	.6	.8	.5	.7	.1	1.4	.9	.4	1.9	.6	.3	.9	.3	.8
-	.3	-	.1	.3	.3	.3	.6	.3	.1	.1	.1	1.0	.4	.8
1.9	1.7	1.7	.9	1.8	.7	2.1	1.6	.7	1.4	.6	1.1	.5	.9	.9
2.1	2.0	1.5	.6	1.4	.5	1.4	1.3	.5	.5	.1	.4	.5	.5	.6
1.7	1.1	.1	-	1.7	.4	.5	.5	.2	.9	.1	.2	.5	.5	.6
1.5	1.2	-	.5	.9	.6	.9	.6	1.2	.1	.1	.4	.7	.6	-
1.5	-	-	.2	.5	.2	-	.3	-	.7	.3	.3	.5	.2	-
1.4	1.7	1.3	1.0	1.1	.7	1.6	.8	.6	1.3	1.4	.9	1.6	1.3	.7
.8	1.2	1.4	.8	.5	1.1	1.7	1.2	.3	.7	.7	.7	1.0	1.0	.9
.7	1.8	.1	.6	.7	.8	.7	.8	.6	.7	.8	1.1	.7	.9	.9
1.0	1.0	.1	.7	.3	.5	.5	.7	.2	.8	.4	.4	.5	.7	.7
.1	.1	.1	.8	-	.1	-	.3	-	.6	.2	.2	.4	.4	.7

Week 2

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
.6	1.7	1.5	1.0	1.7	1.3	1.5	.9	.5	1.0	1.3	.8	.9	1.0	.5
.6	.8	.5	.7	1.4	.6	1.4	.8	.6	1.6	.8	.5	.6	1.0	.6
.2	1.4	.2	.6	.7	.6	1.1	.4	.4	.7	.8	.7	.3	.9	.2
.2	.3	.7	.2	.4	.7	.8	-	.2	1.0	.5	.5	.2	.4	.1
.5	-	.2	-	.2	-	.1	.1	.2	.4	.6	.1	.2	.1	-
1.4	1.4	1.5	.2	1.8	1.8	1.6	1.7	1.3	1.2	.5	1.2	1.4	.7	1.1
1.3	.8	.4	.1	1.5	1.5	1.6	1.2	1.1	.5	.2	1.0	1.2	.6	1.1
.8	.7	.5	.1	1.4	1.2	1.3	1.4	.9	.1	.1	.9	1.2	.7	.6
-	.6	.2	.1	.8	.1	1.0	.1	.2	.1	.1	.6	.8	.6	.4
-	-	.2	.1	.1	.1	.7	.1	.1	.1	.1	.1	.6	.3	.1
1.3	1.3	.6	1.7	1.3	1.9	1.2	1.0	.7	2.0	1.6	1.0	1.1	1.2	1.4
1.0	.9	.4	.8	.8	1.5	1.2	.6	.4	1.3	1.2	.5	.9	1.2	1.0
.8	.4	.6	-	1.0	.5	.6	.1	.5	.1	.8	.4	.6	.4	.3
.8	.2	.5	-	.1	.1	.1	-	.1	.1	.3	.1	.4	.4	.2
.2	.2	.3	-	.2	-	-	-	.1	.1	.1	-	-	.2	-

TABLE 29 (continued):

First set of weekly bioassays - raw data.

Week 3

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
.9	1,3	1,1	1.9	1.2	.7	1.2	1.0	1.5	1.8	2.1	1.6	.9	1.2	.6
.6	.8	1.3	1.6	.9	.1	1.5	1.4	1.6	1.3	1.6	1.0	.8	.6	.6
.6	.7	.8	.9	.5	.1	.2	2.2	1.5	1.5	.5	1.5	.6	.2	.3
.5	.4	.6	.2	.4	-	.1	1.6	1.3	.5	-	.8	1.0	.2	.2
.4	.2	.1	1.3	.2	-	-	.5	.1	.1	-	1.0	1.0	-	.1
1.1	1.5	1.7	1.7	1.5	1.4	1.2	1.5	1.4	1.3	.6	.5	.9	1.1	.9
1.2	1.2	1.0	1.4	1.8	1.8	1.5	1.5	.7	1.7	1.0	.5	.9	.7	.8
.7	1.2	.4	.8	1.1	1.5	1.4	1.7	.5	1.8	.4	.6	.7	.5	.7
.4	.6	.2	-	1.1	1.1	.9	.4	.1	.2	.2	.3	.9	.2	.3
.3	.7	.2	-	.4	.9	-	-	.1	.1	.1	.1	1.4	.1	.7
1.2	1.5	1.3	1.6	1.7	1.8	1.6	1.7	1.5	1.3	1.7	1.5	.8	.9	.5
1.3	1.0	1.3	1.5	1.2	1.9	1.2	1.5	1.1	.6	1.5	1.4	.8	.6	.7
.7	1.3	.9	1.4	1.0	.8	1.1	.6	1.4	.5	.7	1.4	.6	.3	.5
.4	1.0	1.0	.1	.1	1.1	.8	.3	1.2	.3	.1	1.2	.3	.7	.5
.1	.1	.7	-	.1	-	.1	.1	1.9	.1	.3	.7	.1	.4	.6

Week 4

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
2.2	1.5	1.9	1.3	1.6	2.9	1.5	2.4	2.8	2.5	2.6	2.8	1.9	1.0	.9
1.7	2.0	1.3	.1	2.6	2.4	1.7	2.1	2.2	1.8	1.6	2.1	1.4	1.4	.8
2.4	1.5	.5	.1	.8	2.3	2.1	2.3	2.6	1.8	.9	2.1	1.5	.1	1.1
1.2	1.4	.1	.1	.3	1.3	1.1	1.1	2.2	1.1	.2	1.5	1.1	.1	.8
.1	.4	.2	.1	-	1.3	.1	.1	1.5	.5	.1	1.7	.3	.2	.1
2.6	1.1	2.4	2.1	1.8	2.1	1.9	2.8	1.1	2.5	1.8	2.4	1.7	1.0	1.5
2.4	.2	2.6	2.2	1.1	2.7	.9	2.5	1.1	2.0	1.3	1.5	1.6	.9	.9
2.3	.1	2.6	0.9	.3	2.8	1.1	2.9	.9	1.2	.1	2.8	1.6	1.0	1.1
1.0	-	2.6	1.2	.1	2.3	.8	2.2	.1	.3	.1	2.3	.2	.5	1.2
.3	-	1.5	1.0	-	.1	.3	.1	.3	-	.0	2.3	.5	.7	1.6
2.5	2.3	1.8	2.0	1.6	2.6	2.3	2.6	2.4	2.6	2.7	2.4	1.8	1.6	1.6
2.5	2.0	1.6	0.5	2.2	1.9	2.4	2.1	2.0	1.8	1.0	2.1	.9	1.3	1.3
1.8	2.4	2.1	2.1	2.3	1.6	1.7	2.1	2.0	1.5	.4	.1	1.7	1.8	.4
.4	1.8	1.6	1.0	.3	1.9	.3	1.7	.5	.1	.7	1.6	.6	.7	.8
.3	1.0	.9	.1	.1	.9	-	1.3	.1	-	.1	1.9	.2	.1	-

Week 5

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
2.2	1.9	2.2	2.2	2.0	1.4	1.1	1.5	2.8	2.5	2.0	2.1	.7	1.1	.9
1.1	1.5	1.2	1.9	1.7	.9	1.0	1.3	1.8	1.6	1.4	1.3	.7	.7	.2
1.3	1.2	1.2	1.7	1.5	.6	.1	1.2	.8	.2	1.1	.3	.4	.7	.2
.1	.8	.8	1.3	1.3	.6	.2	1.0	.5	.1	1.0	.1	.3	.2	.2
.2	.6	-	.9	.1	.1	.1	.5	-	-	.8	-	.2	.1	-
1.2	2.2	1.7	2.0	2.1	1.4	2.2	1.7	1.8	.7	2.0	2.2	.8	1.1	1.1
1.8	.9	1.4	1.2	2.0	.3	1.9	1.3	1.5	.7	1.9	1.6	.7	.9	.7
1.2	.7	1.7	1.1	1.6	.1	1.8	1.2	1.3	.5	.1	1.5	.5	.5	.5
.5	.2	.2	.2	1.5	.1	.2	1.1	.7	.2	.1	1.0	.3	.3	.2
.1	.1	.2	.1	.1	-	.1	.6	.4	.1	-	.1	.1	.3	.1
2.6	.8	.8	1.7	2.2	2.5	2.5	1.9	1.0	1.0	2.2	1.8	1.1	1.1	.9
1.2	.6	.4	1.1	2.0	1.9	2.2	1.6	.4	1.0	1.6	1.3	1.0	1.0	.6
1.4	.4	.3	.9	1.6	1.5	1.3	1.2	.2	.6	1.5	.2	.9	.9	.5
.8	.2	.2	.2	1.3	1.4	1.2	.7	.2	.5	1.1	.1	.7	.5	.2
.1	.1	.1	-	.1	-	.3	.1	.1	.5	.5	.1	.7	.3	.1

TABLE 29 (continued):

First set of weekly bioassays - raw data.

Week 6

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
1.4	1.3	2.3	1.6	1.8	2.0	1.9	1.9	1.3	2.4	2.2	1.6	1.5	1.6	1.3
1.3	.9	1.1	1.4	1.2	2.0	1.9	1.3	1.2	1.9	2.0	1.0	1.7	1.1	1.0
1.2	.7	1.1	1.3	1.0	.9	1.6	.1	.9	1.9	1.5	.3	.7	.1	.8
1.1	.5	.5	.8	.4	.4	1.3	.1	.1	1.8	.1	.1	.7	-	.7
.9	-	.3	-	.1	.2	.3	-	.1	1.5	.1	.0	.4	-	.2
1.3	2.0	2.1	2.4	1.8	2.0	2.0	1.1	1.5	1.7	1.9	2.1	.9	1.2	1.3
2.0	2.0	1.0	2.2	1.0	1.1	1.9	1.0	1.1	1.5	1.3	1.8	.7	1.0	1.4
1.0	1.7	.9	1.0	1.0	.7	1.9	.5	.8	1.5	.9	1.8	.5	1.0	.6
.2	.1	.8	.3	.7	.4	1.4	.5	.1	1.3	.7	1.0	.4	.1	.1
.1	.1	.3	.1	.2	.2	1.1	.1	.1	1.2	.4	.7	.2	.1	.1
2.1	1.7	1.7	1.6	1.8	2.4	2.0	2.2	1.5	2.1	1.7	2.2	1.0	.7	1.5
1.7	1.2	.5	1.6	1.2	.6	1.8	1.6	1.5	1.8	.7	1.5	.8	.7	.7
1.4	.9	.4	1.1	.6	.4	1.3	1.6	1.4	2.1	.3	1.4	.7	.6	.4
1.2	.6	.3	1.1	.1	.1	.9	1.0	1.3	.5	.1	.5	.7	.5	.2
.1	.2	.1	.6	-	-	.1	.5	.6	-	.1	.4	.3	.4	.3

Week 7

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
2.4	2.1	2.2	1.9	1.9	1.6	2.3	1.8	2.0	2.7	1.7	2.3	1.6	1.5	1.4
1.6	1.6	2.0	.5	1.7	1.1	2.0	1.1	.8	2.1	.9	1.9	1.5	.9	1.2
.6	2.7	2.0	.4	1.6	1.1	.9	.7	.6	2.0	.9	1.2	1.3	.5	.5
.3	1.4	.4	.3	.1	.7	.1	.6	.4	1.6	.4	.7	1.1	.4	.2
.1	.6	.2	.1	-	.4	.1	-	.1	1.4	.1	.1	1.0	.2	-
2.4	2.1	2.3	1.4	1.9	1.9	1.7	1.5	1.9	2.5	2.1	2.4	1.4	1.7	1.1
.4	1.4	2.2	.9	2.0	1.7	1.6	1.1	1.4	1.6	2.0	2.0	.6	1.5	.9
.1	1.2	1.7	.6	1.3	.8	1.0	.7	1.3	1.2	1.4	1.6	.3	1.1	.9
.1	.3	1.6	.5	.4	.7	.2	-	.4	.4	1.2	1.0	.3	.6	.1
.1	.3	.3	-	-	.1	.1	-	-	.4	1.0	.3	.1	.5	-
2.1	2.4	2.1	2.5	2.3	2.0	1.9	2.5	2.0	2.1	1.8	1.5	1.8	1.3	1.5
1.1	1.6	1.5	2.5	2.4	1.6	1.8	1.6	2.0	.4	1.7	1.4	1.3	1.1	1.1
1.1	1.5	.8	.7	1.8	1.6	1.8	1.5	1.4	.1	1.6	1.3	1.2	.6	1.0
1.1	.7	.7	.4	.6	1.7	.5	1.1	1.4	.1	1.6	.6	1.0	.1	.5
.7	-	.7	-	-	.9	.1	1.0	1.1	-	1.0	.3	.8	-	.2

TABLE 30:

Original bioassays: mean and standard deviation of shoot length; mean and standard deviation of shoot indices.

	TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
Week 0:															
Shoot length: mean	.5	.8	.7	.5	.6	.7	1.1	.4	.4	.6	.6	.3	.7	.5	.3
standard deviation	.3	.3	.4	.3	.3	.5	.4	.3	.3	.3	.3	.2	.4	.2	.1
Shoot indices: mean	.3	.8	.8	.3	.4	.6	.6	.3	.3	.5	.7	.2	.6	.8	.6
Standard deviation	.2	.2	.3	.2	.2	.4	.2	.2	.2	.2	.3	.1	.3	.2	.3
Week 1:															
Shoot length: mean	1.5	1.3	.9	.8	1.0	.6	1.2	.9	.5	1.0	.6	.5	.8	.7	.8
standard deviation	.7	.7	.7	.4	.5	.5	.6	.4	.3	.6	.4	.3	.3	.3	.2
Shoot indices: mean	.8	.9	.7	.5	.6	.5	.6	.6	.4	.6	.5	.4	.7	.8	1.0
standard deviation	.3	.3	.4	.3	.3	.3	.3	.3	.2	.3	.4	.2	.2	.2	.0
Week 2:															
Shoot length: mean	.7	.8	.5	.5	.9	.9	1.0	.7	.5	.7	.6	.6	.7	.6	.6
standard deviation	.4	.5	.4	.5	.6	.7	.5	.6	.4	.6	.5	.4	.4	.4	.4
Shoot indices: mean	.5	.8	.7	.3	.6	.6	.5	.4	.3	.5	.6	.4	.6	.8	.8
standard deviation	.3	.3	.3	.3	.4	.4	.3	.3	.3	.4	.4	.3	.3	.3	.3
Week 3:															
Shoot length: mean	.7	.9	.8	1.2	.9	1.1	1.0	1.2	1.2	.9	.8	.9	.6	.6	.5
standard deviation	.4	.4	.5	.6	.6	.6	.5	.7	.6	.7	.6	.5	.3	.3	.3
Shoot indices: mean	.4	.8	.8	.8	.5	.8	.5	.7	.7	.6	.6	.6	.6	.7	.9
standard deviation	.2	.3	.3	.3	.3	.3	.3	.4	.4	.4	.4	.4	.3	.3	.2

TABLE 30 (continued):

(Original bioassays)

	TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
Week 4:															
Shoot length:mean	1.6	1.4	1.6	1.0	1.2	1.9	1.3	1.9	1.5	1.5	.9	1.9	1.1	.8	1.0
standard deviation	.9	.8	.8	.8	.9	.8	.8	.9	.9	.8	.9	.7	.6	.6	.4
Shoot indices: mean	.7	.8	.9	.6	.6	.9	.7	.8	.7	.8	.6	.9	.8	.8	.9
standard deviation	.4	.3	.3	.4	.4	.2	.3	.3	.4	.3	.4	.2	.3	.4	.2
Week 5:															
Shoot length:mean	1.1	.8	.9	1.1	1.4	1.0	1.1	1.1	1.0	.7	1.2	1.0	.6	.6	.5
standard deviation	.8	.6	.7	.7	.7	.8	.9	.5	.8	.7	.7	.8	.3	.4	.3
Shoot indices: mean	.6	.7	.7	.7	.8	.6	.5	.7	.6	.5	.8	.6	.6	.8	.7
standard deviation	.4	.4	.3	.3	.4	.4	.4	.3	.4	.3	.3	.4	.3	.3	.3
Week 6:															
Shoot length:mean	1.1	1.0	.9	1.2	.9	1.0	1.4	1.0	.9	1.7	.9	1.1	.7	.7	.7
standard deviation	.6	.7	.7	.7	.6	.8	.6	.7	.6	.5	.8	.7	.4	.5	.5
Shoot indices: mean	.7	.8	.8	.8	.6	.6	.8	.6	.6	.9	.7	.7	.6	.8	.8
standard deviation	.3	.3	.3	.3	.3	.4	.3	.4	.4	.2	.4	.4	.3	.4	.3
Week 7:															
Shoot length:mean	.9	1.4	1.4	1.0	1.5	1.2	1.1	1.3	1.2	1.3	1.3	1.2	1.0	.9	.8
standard deviation	.8	.8	.8	.8	.8	.6	.8	.5	.7	.9	.6	.7	.5	.5	.5
Shoot indices: mean	.5	.9	.9	.5	.8	.8	.6	.8	.7	.7	.9	.7	.8	.8	.9
standard deviation	.3	.2	.2	.3	.3	.3	.4	.2	.3	.4	.3	.3	.3	.3	.3

TABLE 31:

Log of 2,4-D concentrations in p.p.m. of weekly bioassays as calculated using original controls for shoot indices.

	Wk 0	Wk 1	Wk2	Wk 3	Wk 4	Wk 5	Wk6	Wk 7
TCB	1.90	0.47	1.41	1.50	0.66	1.09	0.87	1.30
TCM	0.73	0.65	0.98	0.81	0.76	1.16	0.97	0.63
TCT	1.35	1.91	1.88	1.29	0.99	1.79	1.43	0.93
TB	1.76	1.32	1.74	0.73	1.21	0.88	0.80	1.26
TM	1.41	0.91	1.02	1.05	0.89	0.45	1.02	0.43
TT	0.95	1.26	0.84	0.55	0.12	0.84	0.97	0.45
WB	0.83	0.74	0.93	0.97	0.70	0.97	0.48	0.91
WM	1.73	0.84	1.23	0.48	0.13	0.51	0.88	0.38
WT	1.84	1.61	1.66	0.81	0.76	1.14	1.00	0.75
LB	1.14	0.75	1.16	0.91	0.41	1.16	0.07	0.60
LM	1.12	1.52	1.45	1.23	1.35	0.60	1.17	0.38
LT	1.75	1.33	1.24	0.82	0.05	0.87	0.70	0.54
PB	1.41	1.26	1.38	1.59	0.90	1.74	1.48	0.83
PM	7.59	5.61	6.27	8.49	6.93	6.93	7.20	4.92
PT	3.96	0.33	2.36	1.46	0.87	2.97	2.09	1.57

APPENDIX 3: Bioassays

Figure 32: weekly bioassays using original controls, with log. values of 2,4-D concentrations - Thrislington Common.

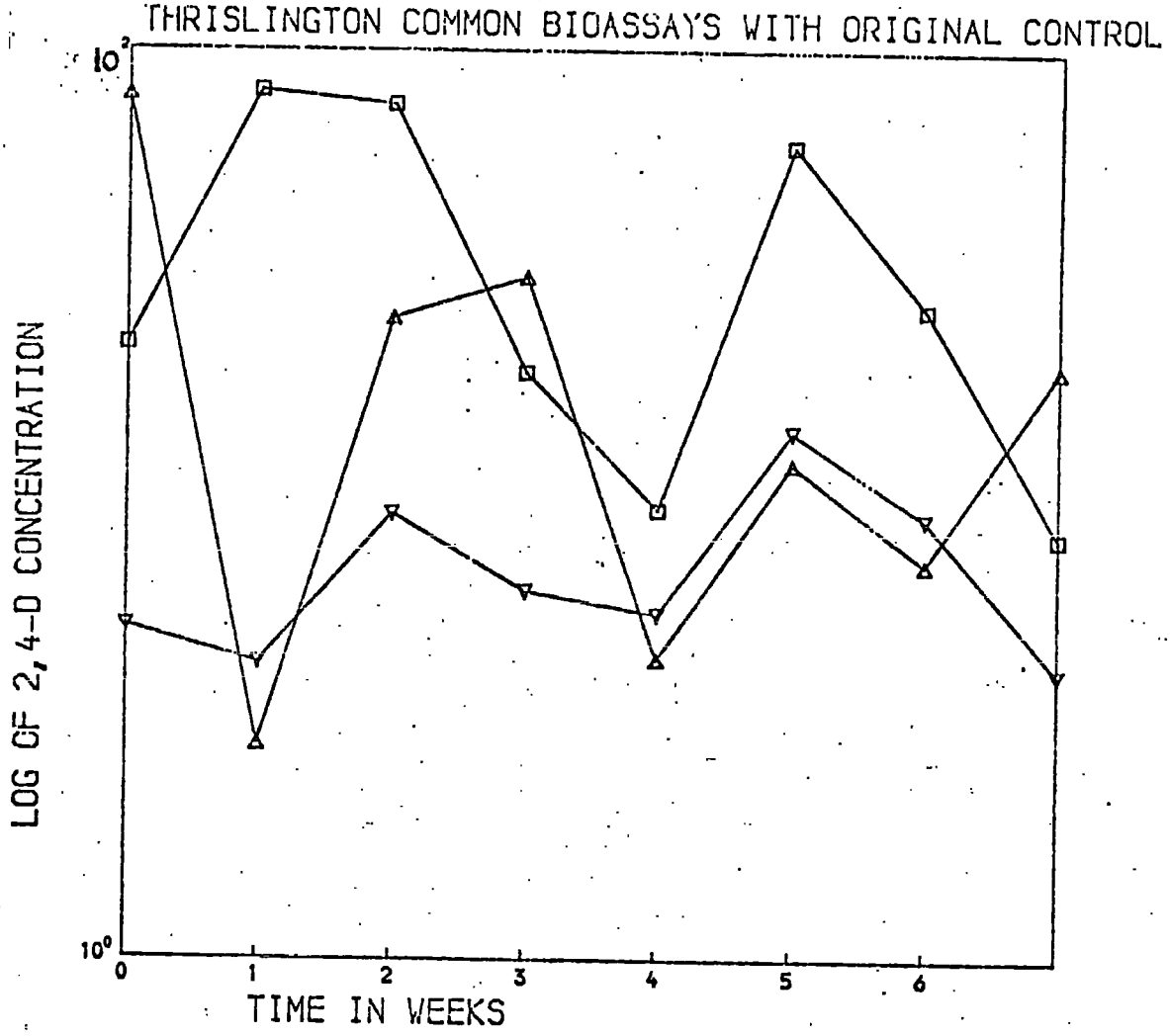

THRISLINGTON COMMON BIOASSAYS WITH ORIGINAL CONTROL
LOG OF 2,4-D CONCENTRATION
10^2
10^0
0
1
2
3
4
5
6
TIME IN WEEKS

Figure 33: weekly bioassays using original controls, with log. values of 2,4-D concentrations - turf.

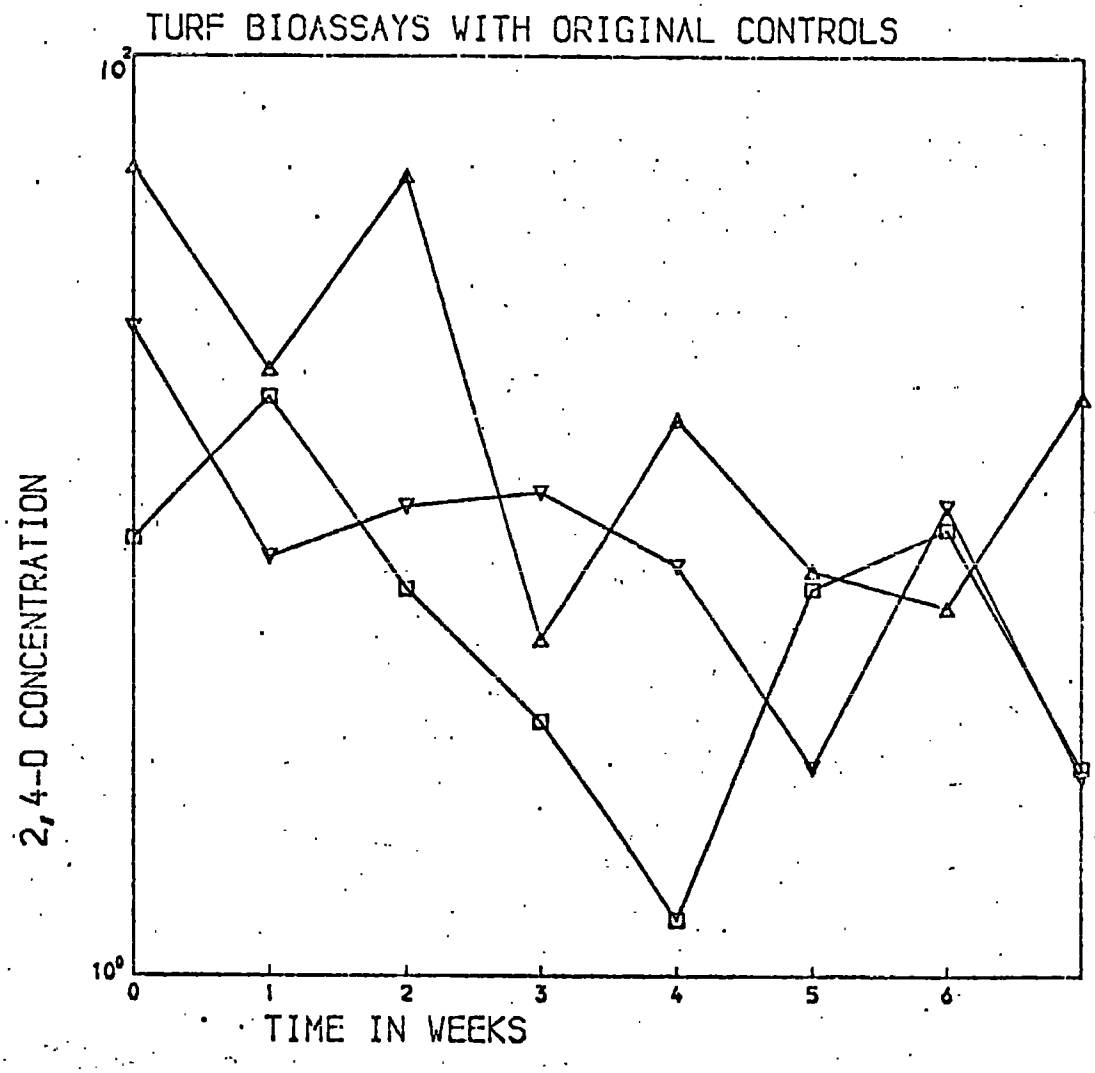

TURF BIOASSAYS WITH ORIGINAL CONTROLS
10^2
10^0
2,4-D CONCENTRATION
0
1
2
3
4
5
6
TIME IN WEEKS

Figure 34: weekly bioassays using original controls, with log. values of 2,4-D concentrations - wood.

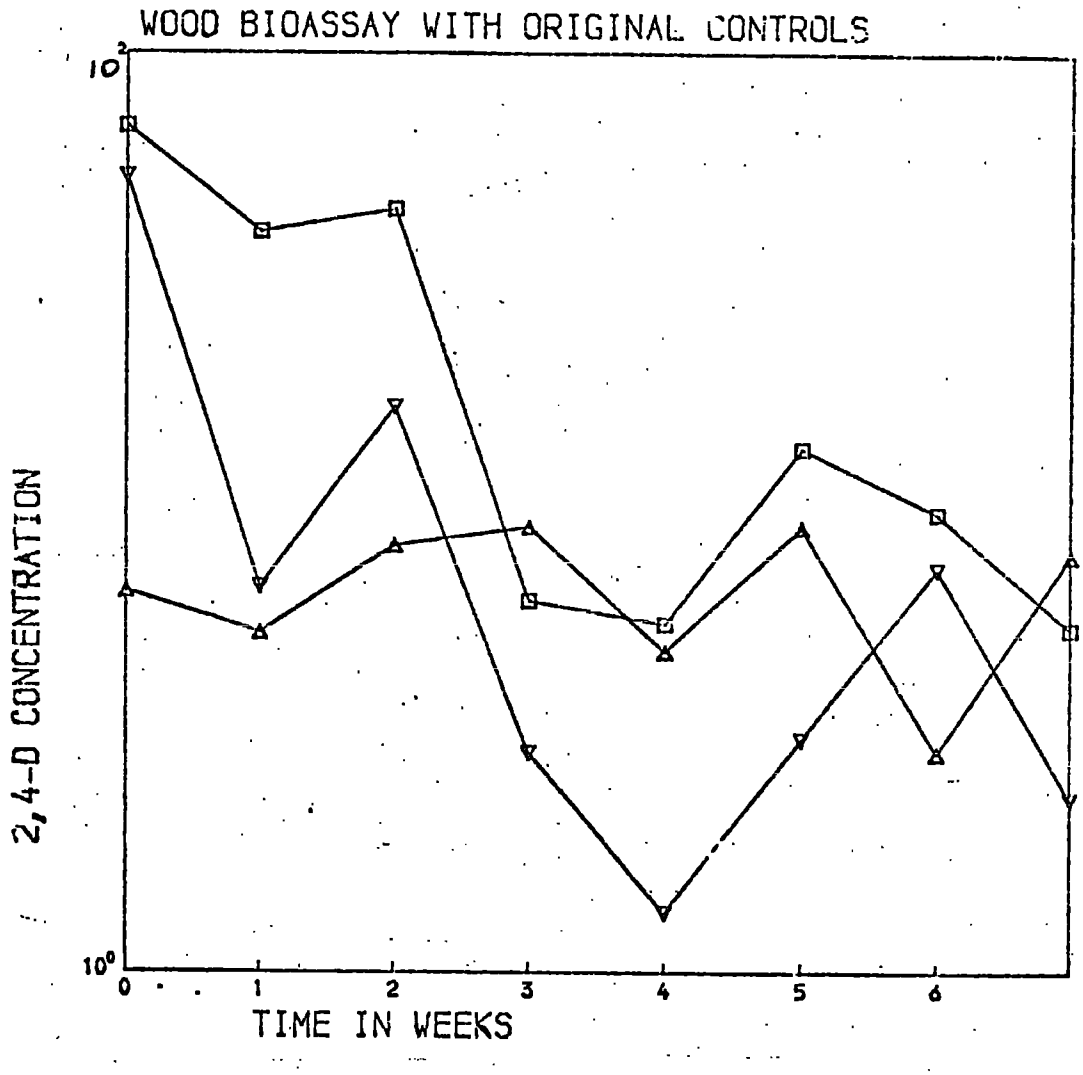

WOOD BIOASSAY WITH ORIGINAL CONTROLS
10^2
10^0
2,4-D CONCENTRATION
0
1
2
3
4
5
6
TIME IN WEEKS

Figure 35: weekly bioassays using original controls, with log. values of 2,4-D concentrations - loam.

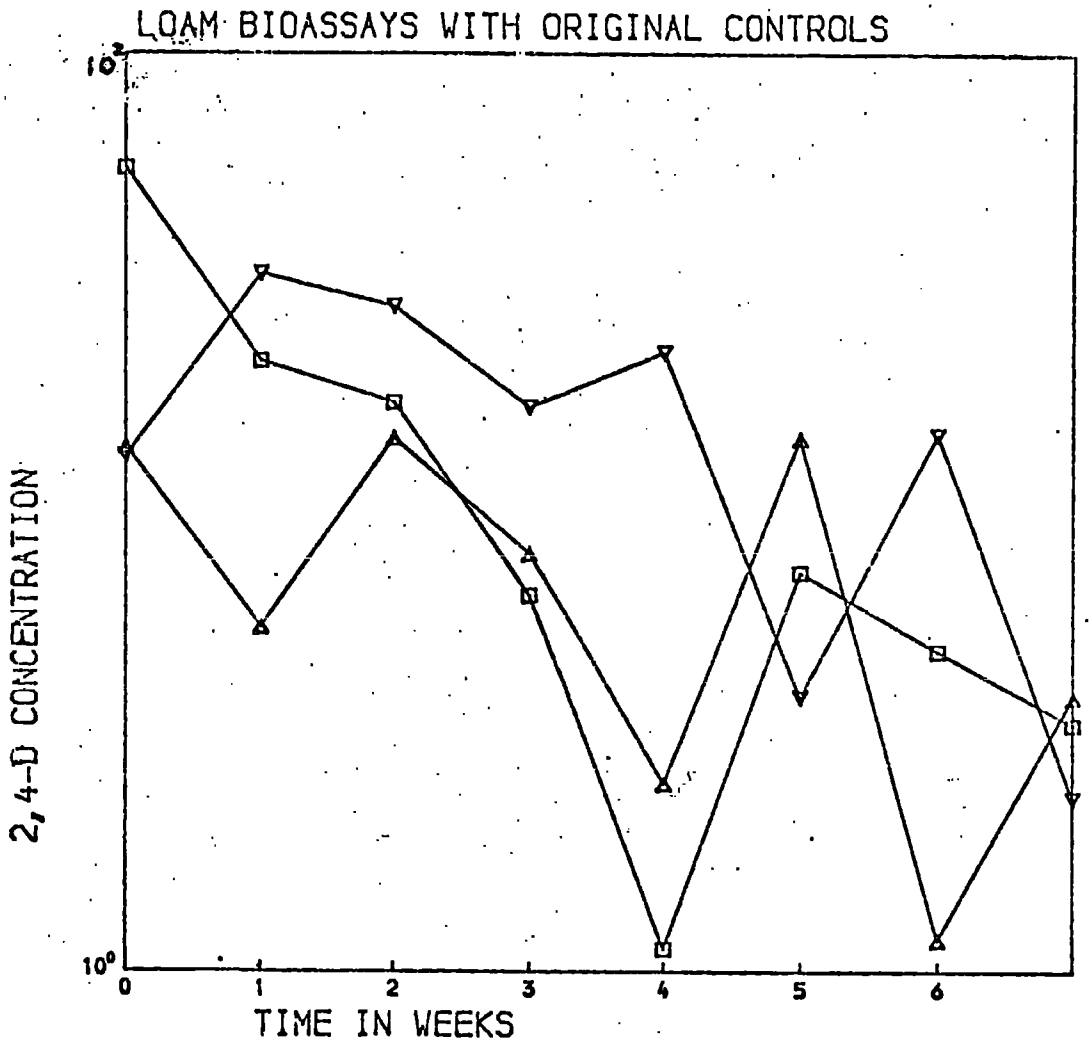

LOAM BIOASSAYS WITH ORIGINAL CONTROLS
10^2
10^0
2,4-D CONCENTRATION
0
1
2
3
4
5
6
TIME IN WEEKS

Figure 36: weekly bioassays using original controls, with log. values of 2,4-D concentrations - peat.

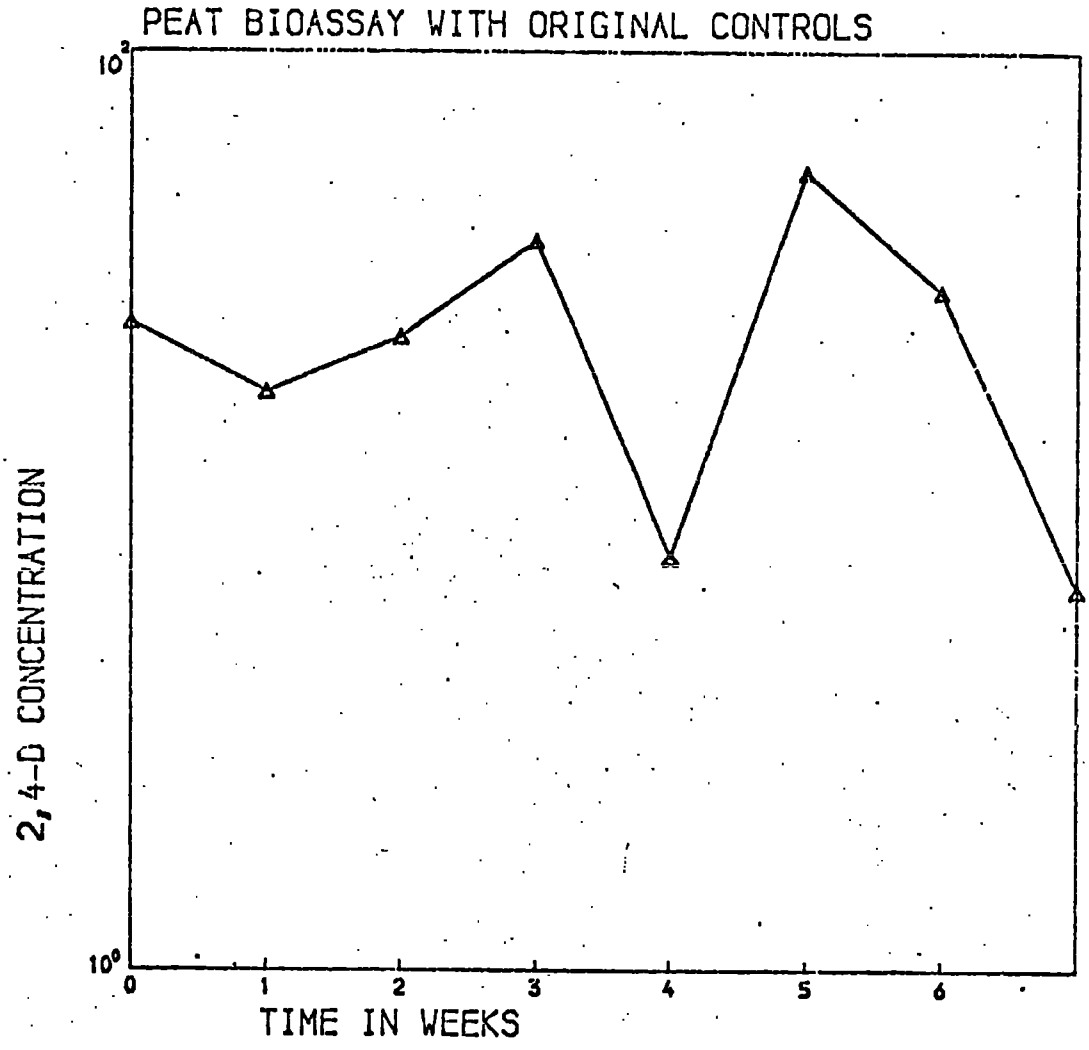

PEAT BIOASSAY WITH ORIGINAL CONTROLS
10^2
10^0
2,4-D CONCENTRATION
0
1
2
3
4
5
6
TIME IN WEEKS

TABLE 32:

Actual 2,4-D concentrations in p.p.m. of weekly bioassay as calculated using original controls for shoot indices.

Soil Type	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7
TCB	79.4	2.9	25.7	31.6	4.6	12.3	7.4	20.0
TCM	5.4	4.5	10.0	6.5	5.8	14.4	9.3	4.3
TCT	22.4	81.3	75.9	19.5	9.8	61.6	26.9	8.5
TB	57.5	20.9	55.0	5.4	16.2	7.6	6.3	18.2
TM	25.7	8.1	10.5	11.2	7.8	2.8	10.5	2.7
TT	8.9	18.2	6.9	3.5	1.3	6.9	9.3	2.8
WB	6.8	5.5	8.5	9.3	5.0	9.3	3.0	8.1
WM	53.7	6.9	17.0	3.0	1.3	3.2	7.6	2.4
WT	69.2	40.7	45.7	6.5	5.8	13.8	10.0	5.6
LB	13.8	5.6	14.5	8.1	2.6	14.5	1.2	4.0
LM	13.2	33.1	28.2	17.0	22.4	4.0	14.8	2.4
LT	56.2	21.4	17.4	6.6	1.1	7.4	5.0	3.5
PB	25.7	18.2	24.0	38.9	7.9	55.0	30.2	6.8

TABLE 33:

Attempts to draw straight lines through the parts of the graphs of actual 2,4-D concentration (based on original controls) against time in weeks where 2,4-D concentration is declining.

Best straight line fit given by

$$y = A_o + A_1 x$$

where y is 2,4-D concentration and x is time in weeks, the fit is significant (less than 0.1) in all cases.

Soil type	Number of points	A_o	A_1	Coefficient of correlation (r)
TCB	4	53.03	-12.07	0.49
TCT	7	52.34	- 3.29	0.24
TB	3	45.72	- 1.25	0.06
WT	3	63.62	-11.75	0.77
LM	5	22.32	0.23	0.044

TABLE 34:

Controls

Shoot length of wheat in cm.

Bioassay week 4 Controls Raw data

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
.3	2.2	2.7	2.7	2.5	2.2	3.0	2.7	2.1	2.6	2.7	2.3	.9	1.4	1.0
.5	3.1	2.5	2.1	2.8	2.4	2.4	2.4	.4	2.8	2.3	2.4	.9	1.3	.5
2.2	1.4	2.4	1.8	2.8	1.7	1.8	1.9	2.3	2.4	1.5	2.5	.6	1.2	.1
2.8	.5	2.4	.1	1.4	1.5	.4	.1	1.4	2.3	1.6	1.9	.6	1.1	.1
1.0	-	.9	.1	.8	.9	.2	.1	-	.1	.1	1.7	.6	.1	.1
2.4	2.5	1.6	-	-	-	1.7	1.9	2.2	-	-	-	1.4	1.2	1.3
1.7	2.0	1.4	-	-	-	.8	1.4	1.6	-	-	-	1.0	.9	1.2
1.2	2.2	.6	-	-	-	.2	.1	1.5	-	-	-	1.0	.8	1.2
.8	2.0	.4	-	-	-	.1	-	.7	-	-	-	.8	.5	.9
.5	1.5	.8	-	-	-	.1	-	.5	-	-	-	.7	.5	.6
2.1	1.8	2.4	-	-	-	2.1	2.0	-	-	-	-	1.1	1.2	1.0
.3	1.6	2.0	-	-	-	1.8	1.4	-	-	-	-	.6	.5	1.5
.1	1.1	2.1	-	-	-	1.8	1.1	-	-	-	-	.9	.7	.7
-	.6	1.6	-	-	-	1.4	.1	-	-	-	-	.3	.7	.1
-	.1	1.9	-	-	-	.1	-	-	-	-	-	.2	.1	.1

Bioassay week 5 Controls Raw data

TCB	TCT	TCM	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
1.9	2.2	1.6	1.7	2.0	1.7	2.1	1.0	1.6	1.7	1.9	1.4	1.0	1.0	1.3
.9	2.1	1.9	2.3	1.8	1.8	1.6	.3	1.8	1.5	1.8	1.3	.9	.9	1.2
.1	1.7	1.7	1.1	.7	1.9	1.6	.1	1.6	1.5	1.4	.8	.3	.3	1.0
.1	1.7	1.3	1.1	.6	1.1	.6	.1	1.1	.6	.5	.4	.2	.3	.5
-	.1	-	.7	.5	-	.2	-	1.2	.1	.5	-	.2	.1	.4
1.1	1.3	1.6	-	-	-	1.6	1.5	1.7	-	-	-	1.3	.7	1.5
1.1	1.2	1.4	-	-	-	1.8	1.1	1.4	-	-	-	1.0	.7	1.0
.8	1.0	.9	-	-	-	.8	.2	1.3	-	-	-	.9	.5	1.0
.6	1.0	.2	-	-	-	1.1	.1	1.1	-	-	-	.6	.6	.2
-	.9	.2	-	-	-	.5	.1	1.1	-	-	-	.6	.3	.0

Bioassay week 6 Controls Raw data

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
2.2	1.2	1.9	1.9	1.7	.8	.5	.7	2.0	1.9	1.0	1.6	1.2	1.0	1.6
1.4	1.1	.7	1.7	1.7	.6	.4	.9	2.1	1.0	1.3	.9	.8	.9	1.1
1.0	.9	.9	1.7	1.1	.5	.3	.5	2.0	.8	1.3	.7	.6	1.2	.9
1.3	.7	.3	.9	.2	.1	.1	.1	.6	1.2	.1	1.1	.4	.9	.9
-	.2	.1	.5	-	.1	-	-	-	.1	-	.3	.1	.5	.6
2.5	1.7	1.0	-	-	-	2.6	2.2	2.4	-	-	-	1.4	1.1	1.2
2.5	1.6	.7	-	-	-	1.8	2.0	2.0	-	-	-	1.2	1.1	1.1
1.4	1.5	.3	-	-	-	1.0	1.5	1.8	-	-	-	1.1	.7	.7
.4	.9	.2	-	-	-	.1	-	1.7	-	-	-	1.1	.1	.9
.3	.7	.2	-	-	-	.1	-	.3	-	-	-	.3	.1	-

Bioassay week 7 Controls Raw data

TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
2.0	2.0	2.2	1.9	1.5	1.8	1.8	1.5	2.2	1.4	1.9	1.4	1.2	1.0	.8
1.3	1.8	1.9	1.5	1.4	1.2	1.3	1.4	1.8	1.3	1.1	1.0	.8	.8	.6
.4	.6	1.5	1.3	1.0	1.0	.6	1.0	1.7	1.1	1.1	.7	.6	.8	.4
.3	.6	1.1	.9	.8	.7	.1	.9	1.4	.7	1.0	.6	.1	.2	.4
-	.5	.8	.7	.3	.3	-	.2	.1	-	.2	-	.1	.1	.1
1.1	2.3	1.8	-	-	-	1.9	1.8	2.0	-	-	-	1.4	.8	.8
.6	1.0	1.2	-	-	-	1.8	1.1	1.9	-	-	-	.6	.8	.7
.5	.9	.6	-	-	-	1.7	.6	1.6	-	-	-	.4	.7	.6
.3	.4	.5	-	-	-	1.7	.4	1.6	-	-	-	.3	.7	.5
-	.3	.4	-	-	-	-	-	-	-	-	-	.1	.7	.2

TABLE 35:

Controls - mean and standard deviations and shoot indices means and standard deviations.

		TCB	TCM	TCT	TB	TM	TT	WB	WM	WT	LB	LM	LT	PB	PM	PT
Week 4	Mean	1.2	1.6	1.7	1.3	2.1	1.7	1.2	1.2	1.4	2.0	1.6	2.2	.8	.8	.7
	standard deviation	.9	.8	.7	1.1	.9	.6	1.0	1.0	.7	1.1	1.0	.3	.3	.4	.5
Week 5	Mean	.8	1.3	1.2	1.4	1.1	1.6	1.2	.5	1.4	1.1	1.2	1.0	.7	.5	.8
	standard deviation	.6	.6	.6	.6	.7	.4	.6	.5	.3	.7	.7	.5	.4	.4	.5
Week 6	Mean	1.4	1.1	.6	1.3	1.2	.4	.8	1.1	1.7	1.0	.9	.5	.8	.8	1.0
	standard deviation	.8	.5	.5	.6	.7	.3	.9	.8	.7	.7	.6	.6	.4	.4	.3
Week 7	Mean	.9	1.0	1.2	1.3	1.0	1.0	1.4	1.0	1.6	1.1	1.1	.9	.6	.7	.5
	standard deviation	.8	.7	.6	.5	.5	.6	.7	.5	.6	.3	.6	.4	.5	.3	.2

TABLE 36:

Second set of weekly bioassays - raw data.

Wheat shoot length in cm.

Week 0:

TCB	TCM	TCT	WB	WM	WT	PB	PM	PT
1.1	2.1	1.5	1.2	2.0	1.7	1.9	1.2	.8
1.3	1.7	.9	1.5	1.8	1.3	1.8	1.0	.7
1.1	1.2	.2	.9	1.9	1.0	1.7	.2	.7
1.0	.7	.1	.6	.4	.7	1.1	.1	.4
.2	.3	.1	-	-	.1	.9	.1	.2
.9	1.3	1.4	.2	2.5	2.1	1.3	1.7	.7
.8	.3	1.2	.1	2.2	1.6	1.2	1.0	.7
.6	.1	1.1	1.6	.2	1.4	.8	1.2	.7
.8	.1	.7	1.8	.1	.9	1.3	.7	.8
.2	-	.7	2.0	.1	.1	.1	.3	.1
.8	2.1	2.4	2.2	1.3	1.0	.8	.9	.8
.9	1.1	1.9	2.3	1.3	1.1	.9	.9	.7
.4	1.6	1.5	2.2	.6	1.0	1.0	1.6	.8
.2	1.4	.4	1.8	.8	.9	1.0	.1	.4
-	1.0	.4	2.4	.8	.8	.4	-	-

Week 1:

TCB	TCM	TCT	WB	WM	WT	PB	PM	PT
1.4	1.1	1.7	1.3	1.1	1.3	1.1	1.0	.6
1.3	.6	1.4	1.1	1.0	2.1	.7	1.0	.4
.6	.6	1.2	1.0	.8	1.0	.3	.6	.1
.9	.6	.9	.5	.7	.9	.5	.1	.1
.6	.5	.1	.1	-	.1	.1	-	.1
1.2	1.9	2.1	1.9	1.1	1.3	1.0	.7	.7
1.1	1.7	2.0	1.2	.4	1.0	1.1	1.0	.9
1.0	1.5	1.7	1.0	.3	.5	.5	.6	.5
.1	.3	1.6	.4	.5	.7	.9	.3	.4
.1	.2	.7	.1	.1	.4	.1	.1	.2
1.2	1.9	1.6	1.5	1.5	.7	1.3	.8	.7
1.1	1.6	.6	1.3	1.6	.9	1.2	.6	.8
1.0	1.6	.3	.9	1.3	.6	.6	.3	.7
.1	.9	.1	.4	1.5	.2	.9	.2	.7
.1	.3	.1	.1	.1	-	.1	-	.4

Week 2:

TCB	TCM	TCT	WB	WM	WT	PB	PM	PT
1.8	1.7	1.8	2.2	1.7	1.5	1.0	1.2	1.0
.6	1.2	1.7	1.8	1.5	1.1	1.0	1.5	.3
.9	.6	1.5	1.8	1.4	1.0	.8	.7	.3
.6	.5	1.5	.5	1.3	.7	.6	.6	.2
-	.2	1.5	.1	.3	.2	.6	.1	.2
1.7	.9	1.7	1.9	1.8	1.6	.8	1.8	1.6
1.6	.2	1.0	1.8	1.4	.7	1.2	1.3	1.1
1.0	.2	.6	1.2	1.3	.7	.9	1.2	.7
.8	.1	.2	.7	.5	.7	.5	.7	.8
-	-	-	.1	-	.2	.8	.5	.4
1.3	1.8	1.5	1.4	1.5	1.0	2.3	1.3	.7
1.0	1.2	.6	1.3	1.5	.9	1.3	1.0	.9
.9	1.0	.4	1.3	1.5	.6	.3	.8	.4
.8	.2	.6	.6	.6	.4	.1	.7	.5
.6	-	.5	.6	.6	.1	.1	.1	-

TABLE 36 (continued):

Second set of weekly bioassays - raw data.

Week 3:

TCB	TCM	TCT	WB	WM	WT	PB	PM	PT
1.8	1.0	1.9	2.3	1.6	.3	1.4	1.2	1.2
.7	.9	.6	2.0	1.6	.2	1.1	1.0	.9
.9	.8	.4	1.9	.5	.1	1.0	.9	.7
.3	.8	.4	1.8	.1	.1	.5	.7	.5
.1	.3	.2	.3	.1	.1	.4	.6	.4
1.9	2.1	1.6	2.4	1.4	1.7	1.3	1.2	.8
1.7	1.0	1.0	2.3	1.3	1.4	1.2	1.2	.8
1.1	.9	1.0	1.1	1.1	1.2	1.0	.6	.7
.8	.9	.3	1.0	.7	1.1	.8	.2	.5
.4	-	-	.9	-	.4	.2	.1	-
1.5	1.6	1.5	2.6	1.3	2.3	1.9	1.0	.8
1.2	1.2	1.2	1.6	1.1	1.9	1.0	.9	.8
.9	.6	1.2	1.5	.9	1.8	.6	.6	.7
.1	.3	1.2	1.4	.8	1.5	.3	.1	.6
-	.1	.7	.2	-	.4	.1	-	.5

Weeks 4 - 7 as for first set of bioassays.

TABLE 37:

Second set of weekly bioassays. Mean and standard deviation of wheat shoot length in cm; mean and standard deviation of shoot indices calculated from weekly controls.

	TCB	TCM	TCT	WB	WM	WT	PB	PM	PT
Week 0									
Shoot length mean	.7	1.1	1.0	1.5	1.1	1.0	1.1	.8	.6
standard deviation	.4	.7	.7	.8	.8	.5	.5	.6	.2
Shoot index mean	.6	.6	.5	.8	.7	.7	.9	.7	.8
standard deviation	.3	.4	.3	.3	.4	.3	.2	.4	.3
Week 1									
Shoot length mean	.8	1.0	1.1	.9	.9	.8	.7	.6	.5
standard deviation	.5	.6	.7	.6	.5	.5	.4	.3	.3
Shoot index mean	.7	.7	.7	.6	.8	.6	.7	.8	.6
standard deviation	.4	.3	.4	.4	.3	.3	.4	.3	.3
Week 2									
Shoot length mean	1.0	.8	1.1	1.2	1.2	.8	.8	.9	.7
standard deviation	.4	.6	.6	.7	.5	.4	.5	.5	.4
Shoot index mean	.7	.6	.9	.8	.8	.5	.8	.8	.6
standard deviation	.2	.4	.2	.3	.3	.3	.3	.3	.3
Week 3									
Shoot length mean	1.0	.9	.9	1.6	1.0	1.0	.9	.7	.7
standard deviation	.6	.5	.5	.7	.5	.8	.5	.4	.2
Shoot index mean	.7	.7	.7	.8	.8	.6	.8	.9	1.0
standard deviation	.4	.3	.3	.3	.3	.4	.3	.2	.06
Week 4									
Shoot length mean	1.6	1.4	1.6	1.3	1.9	1.5	1.1	.8	1.0
standard deviation	.9	.8	.8	.8	.9	.9	.6	.6	.4
Shoot index mean	.8	.7	.8	.8	.9	.7	.8	.7	.9
standard deviation	.4	.4	.3	.3	.3	.4	.3	.4	.2

TABLE 37 (continued):

Second set of weekly bioassays. Means and standard deviations of shoot lengths and indices.

	TCB	TCM	TCT	WB	WM	WT	PB	PM	PT
Week 5									
Shoot length mean	1.1	.8	1.2	1.1	1.1	1.0	.6	.6	.5
standard deviation	.8	.6	.7	.9	.5	.8	.3	.4	.3
Shoot index mean	.7	.5	.6	.6	.9	.6	.8	.8	.5
standard deviation	.4	.3	.4	.4	.2	.4	.3	.3	.3
Week 6									
Shoot length mean	1.1	1.0	.9	1.4	1.0	.9	.7	.7	.7
standard deviation	.6	.7	.7	.6	.7	.6	.4	.5	.5
Shoot index mean	.7	.7	.8	.9	.7	.5	.8	.7	.6
standard deviation	.3	.4	.3	.3	.4	.3	.3	.4	.4
Week 7									
Shoot length mean	.9	1.4	1.4	1.1	1.3	1.2	1.0	.9	.8
standard deviation	.8	.8	.8	.8	.5	.7	.5	.5	.5
Shoot index mean	.6	.8	.8	.6	.9	.7	.9	.9	.8
standard deviation	.4	.3	.3	.4	.1	.3	.3	.2	.3

TABLE 38:

Log of 2,4-D concentrations in p.p.m. of weekly bioassays using weekly controls for shoot indices.

	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7
TCB	1.03	0.67	0.80	0.62	0.53	0.63	0.71	0.92
TCM	1.41	1.28	1.48	1.05	1.08	1.65	1.16	0.73
TCT	2.97	2.11	0.92	2.03	1.69	2.55	1.55	1.65
WB	0.33	0.72	0.33	0.30	0.44	0.73	0.16	0.79
WM	0.62	0.14	0.14	0.31	0.07	-0.15	0.62	-0.07
WT	0.85	1.14	1.39	1.17	0.76	1.12	1.19	0.86
LB	0.33	0.99	0.87	0.59	0.68	0.88	0.91	0.44
LM	9.18	7.53	5.31	3.69	8.37	6.12	9.00	2.28
LT	1.82	3.85	3.79	0.50	1.12	4.46	3.73	1.76

TABLE 39:

Actual 2,4-D concentrations in p.p.m. of weekly bioassays as calculated using original controls for shoot indices.

Soil type	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
TCB	10.7	4.7	6.3	4.2	3.4	4.3	5.1	8.3
TCM	25.7	19.1	30.2	11.2	12.0	44.7	14.5	5.4
TCT	Significance of conversion graph too low							
WB	2.1	5.2	2.1	2.0	2.8	5.4	1.4	6.2
WM	Significance of conversion graph too low							
WT	7.1	13.8	24.5	14.8	5.8	13.2	15.5	7.2
PB	2.1	9.8	7.4	3.9	4.8	7.6	8.1	2.8
PM	Significance of conversion graph too low							
PT	Significance of conversion graph too low							

TABLE 40:

Comparison of wet and dry soils - raw data.

Wheat shoot length in cm.

Week 0

Wet soil			Dry soil		
TCB	TCM	TCT	TCB	TCM	TCT
1.4	1.0	.6	.3	1.4	1.2
1.0	.9	1.2	.4	1.0	1.2
.9	.9	1.2	.2	1.0	.2
.2	.7	.1	.2	.6	.1
.5	-	-	-	.1	.1
.8	1.0	1.0	.8	1.8	.9
.5	1.0	1.0	.3	.9	.7
.5	1.0	1.0	.5	.4	.6
.1	.2	.7	.5	1.0	.3
.1	.1	.1	-	.6	.2
1.6	1.2	1.2	.6	1.3	1.2
1.4	1.2	1.0	.4	.6	1.1
1.1	.9	.9	.2	.7	1.3
.1	.6	.4	.2	.2	.1
-	.3	.2	.1	.1	.1

Week 1

Wet Soil			Dry soil		
TCB	TCM	TCT	TCB	TCM	TCT
1.6	1.4	1.9	1.5	1.5	2.9
1.8	1.4	1.9	1.5	1.8	1.2
.9	1.1	1.2	1.8	1.0	1.0
1.7	.1	1.0	.8	1.1	.9
1.7	.1	.3	-	-	1.1
.8	1.7	.8	.9	2.1	1.8
.9	1.5	.8	.9	1.1	1.2
.5	1.1	.7	.3	.9	1.0
.3	.3	.6	.2	.8	.7
.1	.2	.1	.1	.1	.6
1.5	1.3	1.8	1.4	2.1	2.2
.9	1.4	2.0	1.4	1.5	1.8
1.0	1.3	.9	1.1	.7	.6
.6	.6	1.5	1.0	.1	.8
.1	.2	.7	1.0	-	.2

TABLE 40 (continued):

Comparison of wet and dry soils - raw data.

Week 2

Wet soil			Dry soil		
TCB	TCM	TCT	TCB	TCM	TCT
1.6	1.5	1.5	1.2	2.4	2.0
.7	.8	1.3	1.1	2.1	1.4
.6	.6	.6	.6	.9	1.4
.5	.6	.3	.7	.5	.6
.2	.5	-	.3	.1	.5
1.0	.8	1.0	.9	2.0	1.5
1.2	.6	.7	.8	1.5	1.1
.2	.5	.6	.6	1.4	.3
.2	.3	.4	1.0	1.3	.2
.2	.3	.1	.2	.1	-
1.1	1.5	1.0	.9	1.7	1.9
.7	.9	.6	.8	1.0	1.9
.7	.6	.4	.7	.7	1.7
.2	.6	.4	.7	.1	1.6
.1	.4	.2	.1	.1	1.5

Week 3

Wet soil			Dry soil		
TCB	TCM	TCT	TCB	TCM	TCT
1.0	1.7	1.6	1.1	1.0	1.3
1.0	1.5	1.4	.7	.9	1.2
.8	1.1	1.1	.6	.1	.6
.7	1.1	1.1	.5	-	.5
.4	-	.6	.5	-	.2
.9	1.4	1.7	.5	1.2	1.9
.6	1.3	1.2	.4	.9	1.8
.5	1.2	1.0	.1	.5	1.2
.5	.7	1.0	.0	.2	.4
.2	.4	.8	-	.1	.0
1.2	1.4	1.9	1.0	1.5	1.1
1.1	1.1	.8	.7	1.1	.6
.6	1.0	.2	.7	1.2	.2
.3	.5	.1	.2	.3	.1
.4	.1	.1	.1	-	.1

TABLE 41:

Comparison of wet and dry soils. Mean and standard deviations of shoot length in cm; mean and standard deviations of shoot indices calculated from original controls.

	Wet			Dry		
	TCB	TCM	TCT	TCB	TCM	TCT
Week 0						
Shoot length : mean	.7	.8	.8	.4	.8	.6
standard deviation	.5	.4	.4	.2	.5	.5
Shoot indices : mean	.4	.8	.8	.2	.5	.5
standard deviation	.3	.3	.3	.1	.3	.4
Week 1						
Shoot length : mean	1.0	.9	1.0	1.0	1.1	1.2
standard deviation	.6	.6	.6	.5	.7	.7
Shoot indices : mean	.6	.7	.9	.7	.7	.8
standard deviation	.3	.4	.2	.3	.3	.3
Week 2						
Shoot length : mean	.6	.7	.7	.7	1.1	1.2
standard deviation	.5	.4	.4	.3	.8	.6
Shoot indices : mean	.4	.7	.8	.5	.6	.8
standard deviation	.3	.2	.3	.2	.4	.3
Week 3						
Shoot length : mean	.7	1.0	1.0	.5	.8	.7
standard deviation	.3	.5	.6	.3	.5	.6
Shoot indices : mean	.4	.9	.8	.4	.5	.5
standard deviation	.2	.3	.3	.2	.3	.4

TABLE 42:

Log. of 2,4-D concentrations in p.p.m. of wet/dry soil comparison, as calculated using original controls for shoot indices.

		Wk 0	Wk 1	Wk 2	Wk 3
WET	TCB	1.44	1.09	1.64	1.52
	TCM	0.84	1.10	1.05	0.68
	TCT	1.42	0.89	1.52	1.25
DRY	TCB	2.06	1.07	1.48	1.81
	TCM	0.47	0.28	0.28	0.53
	TCT	0.41	0.41	0.41	0.41

TABLE 43:

Actual 2,4-D concentration in p.p.m. of wet/dry soil comparison, as calculated using original controls for shoot indices.

		Wk 0	Wk 1	Wk 2	Wk 3
WET	TCB	27.5	12.3	43.7	33.1
	TCM	6.9	12.6	11.2	4.8
	TCT	26.3	7.8	33.1	17.8
DRY	TCB	114.8	11.7	30.2	64.6
	TCM	2.95	1.9	1.9	3.4
	TCT	2.6	2.6	2.6	2.6

TABLE 44:

Log. of 2,4-D concentrations in p.p.m. of wet/dry soil comparisons, as calculated using weekly controls for shoot indices.

		Wk 0	Wk 1	Wk 2	Wk 3
WET	TCB	1.44	0.85	1.26	1.52
	TCM	0.84	1.57	1.70	0.79
	TCT	1.42	2.58	3.07	1.28
DRY	TCB	2.06	0.58	0.32	1.70
	TCM	0.47	1.15	1.54	1.20
	TCT	0.41	2.41	0.48	3.05

TABLE 45:

Actual 2,4-D concentrations in p.p.m. of wet/dry soil comparison, as calculated using weekly controls for shoot indices.

	Soil type	Wk 0	Wk 1	Wk 2	Wk 3	
Wet	TCB	27.5	7.1	18.2	33.1	
	TCM	6.9	37.2	50.1	6.2	
	TCT	Conversion graph not sufficiently significant				
Dry	TCB	114.8	3.8	2.1	50.1	
	TCM	3.0	14.1	34.7	15.8	
	TCT	Conversion graph not sufficiently significant				

Figure 37: comparison of wet and dry soil from Thrislington Common: wet soil using weekly controls for calculations.

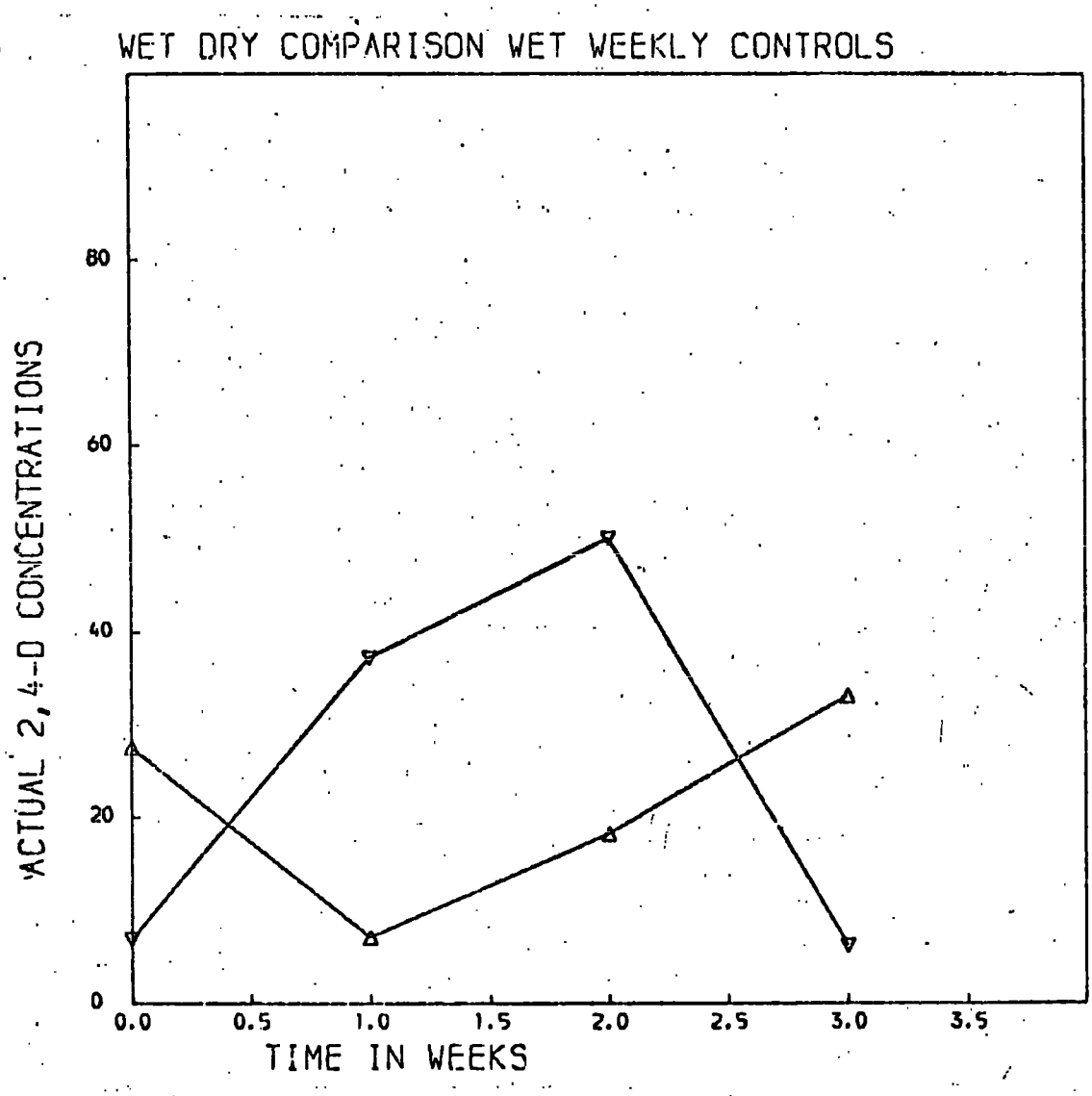

WET DRY COMPARISON WET WEEKLY CONTROLS
ACTUAL 2,4-D CONCENTRATIONS
0
20
40
60
80
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
TIME IN WEEKS

Figure 38: comparison of wet and dry soils from Thrislington Common: dry soil using weekly controls for calculations.

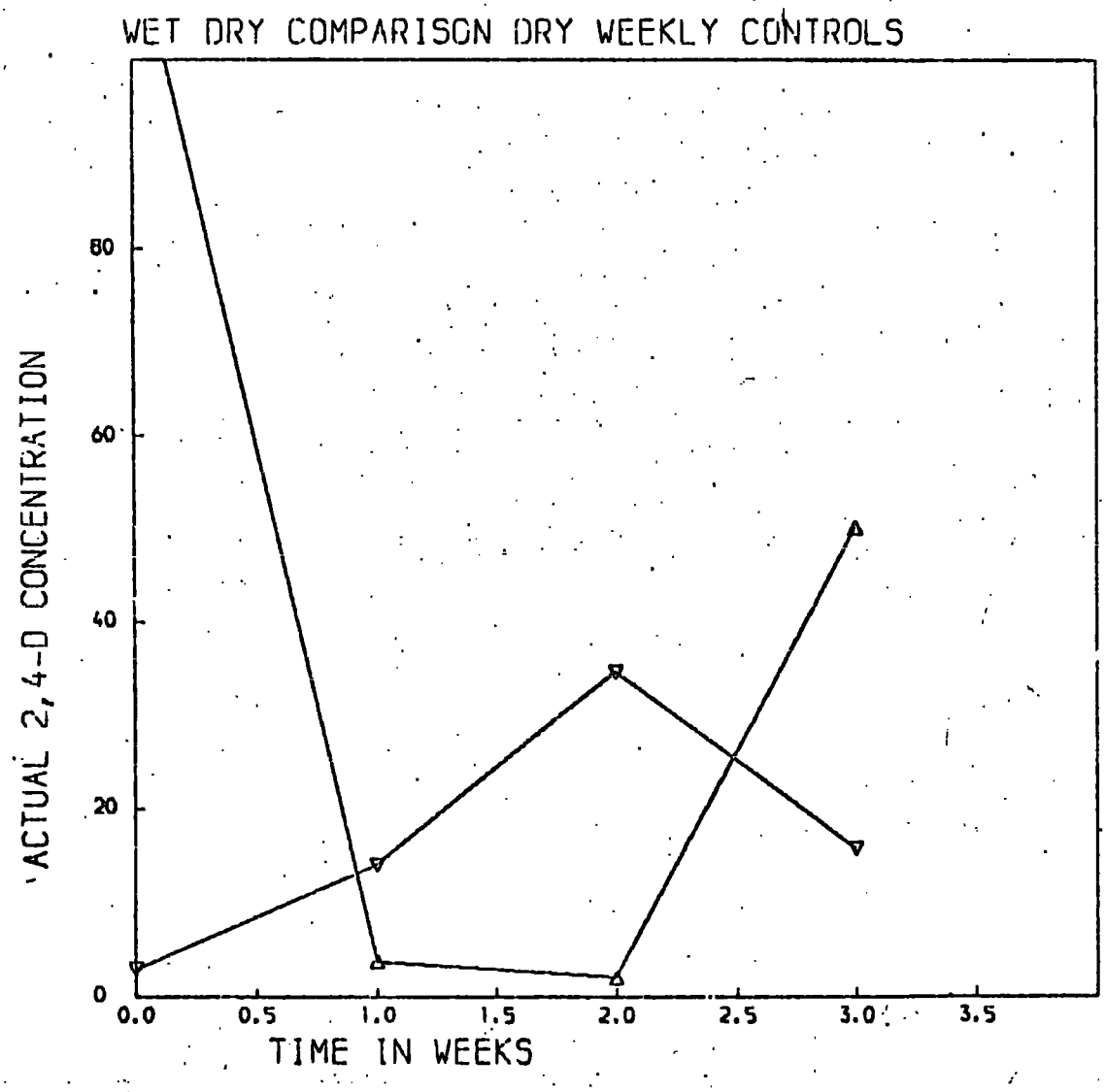

WET DRY COMPARISON DRY WEEKLY CONTROLS
ACTUAL 2,4-D CONCENTRATION
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
TIME IN WEEKS

TABLE 46:

Bioassay results for 100ppm 2,4-D solution left in containers in greenhouse on 25th June.

	Week 0	Week 1	Week 2
Wheat shoot growth in cm.	.5	.2	.1
	.2	.1	.1
	.2	.1	.1
	.2	.2	.1
	.0	.2	.1
	.3	.2	.1
	.6	.2	.1
	.2	.1	.1
	.2	.0	.1
	-	.1	.1
	.4	.1	.1
	.1	.1	.1
	.2	.2	.1
	.2	.1	.1
	.3	.1	.1
Mean	.3	.13	.1
Standard deviation	.14	.06	.0

TABLE 47:

Results from Thrislington Common field bioassays.

1st spraying - 31st May

Bioassay 1 Ungerminated wheat seeds set up on 7th June; shoot measured 12th June.

	Control (unsprayed)	Sample1 (sprayed)	Sample 2 (sprayed)	Sample 3 (sprayed)	Sample 4 (sprayed)
Length of shoot in cm.	1.1 .4 .6	.5 .0 .5 1.0 .5	.0 .0 .0 .0 .0	.1 .0 .0 .0 .4	.9 .8 .8 .7 .05
Mean length in cm.	.7	.5	.0	.1	.7
Standard deviation	.4	.4	.0	.2	.3
Mean length in cm of sprayed samples taken together			.3		
Standard deviation			.4		

Bioassay 2 Wheat and cucumber seeds set to germinate on 13th June, set up on soils for bioassay on 15th June, and measured on 17th June. The growth in cm. of the wheat shoot and cucumber radicle between 15th and 17th June were measured.

	Control (unsprayed)	Sample 1 (sprayed)	Sample 2 (sprayed)	Sample 3 (sprayed)
Wheat shoot - growth in cm.	1.6 .9	2.0 .6	1.7 .4	3.5 2.0
Mean of wheat shoot growth in cm.	1.3	1.3	1.1	2.8
Standard deviation	.5	1.0	.9	1.1
Mean of growth in cm. of sprayed samples taken together			1.7	
Standard deviation			1.1	
Cucumber radicle - growth in cm.	5.2 3.7	1.9 .5	- -	4.9 1.3
Mean of cucumber growth	4.5	1.2	-	3.1
Standard deviation	1.1	1.0	-	2.5
Mean growth in cm. of sprayed samples taken together			2.1	
Standard deviation			1.9	

TABLE 47 (continued):

Results from Thrislington Common field bioassays.

In the following bioassays in this Appendix ungerminated wheat seeds were placed on the soils to be assessed, and the growth in cm. of the shoot measured after 3 days.

2nd Spraying - 27th June

Bioassay 1 Seeds measured on 8th July.

	Control (unsprayed)	Control (unsprayed)	Sample 1 (sprayed)	Sample 2 (sprayed)	Sample 3 (sprayed)
Length of wheat shoot in cm.	3.1	1.0	1.4	1.6	1.5
	2.0	1.7	1.7	1.6	.4
	2.1	2.1	1.7	1.6	.6
	1.7	1.2	.5	.2	.4
	1.2	-	.8	.3	-
Mean	2.0	1.5	1.2	1.1	.7
Standard deviation	.7	.5	.5	.7	.5
Means of controls, or sprayed samples taken together	1.8		1.0		
Standard deviation	.6		.6		

Bioassay 2 Seeds measured on 15th July.

	Control	Control	Sample 1	Sample 2	Sample 3
Length of wheat shoot in cm.	1.6	1.4	2.1	1.9	1.7
	.9	1.3	1.9	1.4	.5
	.6	.2	1.4	.9	.2
	.5	-	1.2	.2	.1
	.5	-	.8	.2	.1
Mean	.8	1.0	1.5	.9	.5
Standard deviation	.5	.7	.5	.7	.7
Means of controls, or sprayed samples taken together	.9		1.0		
Standard deviation	.5		.7		

Bioassay 3 Seeds measured on 23rd July.

	Control	Control	Sample 1	Sample 2	Sample 3
Length of wheat shoot in cm.	1.0	1.5	1.3	.6	.2
	1.3	1.2	1.2	.8	.5
	.8	1.2	.5	.3	.5
	1.2	.6	.4	.2	.4
	.2	.4	.5	.1	.4
Mean	.9	1.0	.8	.4	.4
Standard deviation	.4	.5	.4	.2	.1
Means of controls, or sprayed samples taken together	.9		.5		
Standard deviation	.4		.3		

TABLE 47 (continued):

Results from Thrislington Common field bioassays
2nd Spraying - 27th June - continued.

Bioassay 4 Seeds measured 30th July.

	Control	Control	Sample 1	Sample 2
Length of wheat shoot in cm.	1.0 .1 - - -	2.4 2.0 .1 .1 .1	2.3 2.6 1.8 .1 .1	1.4 1.9 .9 .9 .1
Mean Standard deviation	.6 .6	.9 1.2	1.4 1.2	1.0 .7
Mean of controls, or sprayed samples taken together Standard deviation	.8 1.0		1.2 .9	

3rd Spraying - 27th July.

Bioassay 1 Seeds measured 6th August.

	Control	Control	Sample 1	Sample 2	Sample 3
Length of wheat shoot in cm.	1.7 1.2 1.1 1.1 .7	1.8 1.5 1.4 1.3 .5	1.5 .7 .7 .1 -	1.7 1.4 1.1 .5 .0	1.4 1.1 1.1 .3 .2
Mean Standard deviation	1.2 .4	1.3 .5	.8 .6	.9 .7	.8 .5
Mean of controls, or sprayed samples taken together Standard deviation	1.2 .4		.8 .6		

Bioassay 2 Seeds measured 13th August.

	Control	Control	Sample 1	Sample 2	Sample 3
Length of wheat shoot in cm.	2.1 1.8 1.1 .9 .9	2.3 2.0 1.4 1.1 .5	2.1 2.0 1.8 1.7 .1	2.1 1.8 1.9 .2 .1	1.4 1.8 1.0 2.1 .9
Mean Standard deviation	1.4 .6	1.5 .7	1.5 .8	1.2 1.0	1.4 .5
Mean of controls, or sprayed samples taken together Standard deviation	1.4 .6		1.4 .8		

APPENDIX 4: radio-chemical experiments.

TABLE 48:

Radio-chemical comparison of uptake of 2,4-D by previously ungerminated wheat seeds on top soil from Thrislington Common and on top peat.

Hours after experiment started	Live seeds on Thrislington Common top soil			Live seeds on peat top soil		
	Uptake in Counts per minute	% accuracy	S	Uptake in Counts per minute	%accuracy	S
.5	358.8	5.0	.589	65.2	15.0	.614
1.0	682.2	5.0	.537	83.0	10.0	.681
1.5	671.8	5.0	.673	66.0	15.0	.518
2.0	559.6	5.0	.714	87.2	10.0	.684
2.5	1184.0	3.0	.669	70.4	15.0	.646
3.0	1379.6	3.0	.674	73.4	15.0	.672
3.5	924.6	3.0	.713	82.4	10.0	.680
4.0	1224.0	3.0	.483	63.4	15.0	.514
6.0	1008.0	3.0	.707	89.0	10.0	.700
8.0	3139.6	2.0	.681	101.6	10.0	.687
10.0	1193.0	3.0	.661	137.2	10.0	.705
12.0	1343.8	3.0	.706	125.4	10.0	.664
16.0	3845.6	2.0	.672	106.0	10.0	.673
22.0	3282.4	2.0	.684	140.6	10.0	.684
28.0	1705.8	3.0	.765	156.4	10.0	.702
36.0	2197.6	2.0	.689	142.2	10.0	.684
46.0	1987.6	3.0	.597	458.6	5.0	.654
58.0	1542.6	3.0	.632	418.0	5.0	.639
70.0	2989.8	2.0	.465	667.4	5.0	.672

Figure 39: uptake of radio-chemically labelled 2,4-D by live seeds on Thrislington Common and peat top soils.

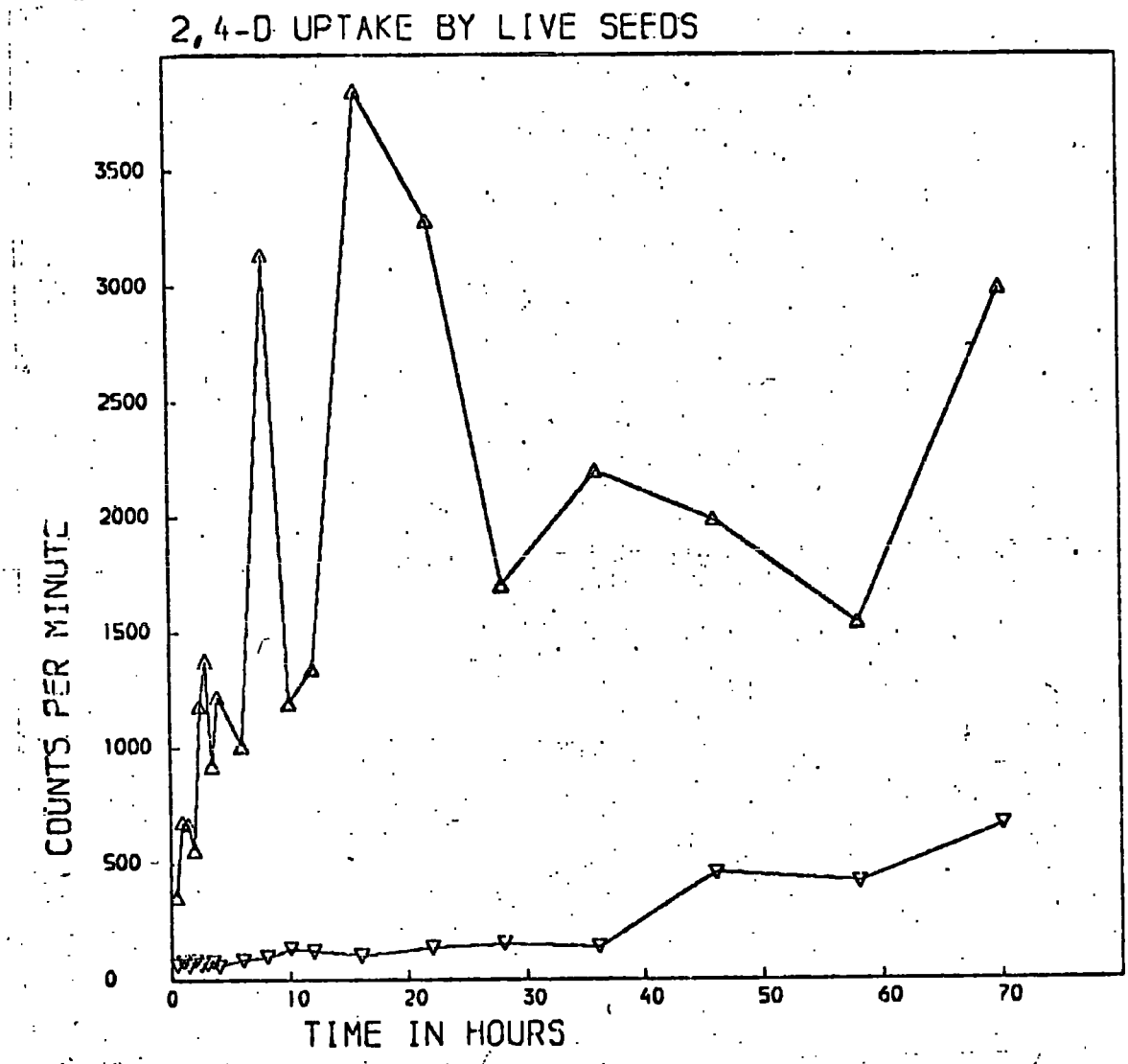

2,4-D UPTAKE BY LIVE SEEDS
COUNTS PER MINUTE
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
70
TIME IN HOURS

TABLE 49:

Radio-chemical comparison of uptake of 2,4-D by previously ungerminated wheat seeds on top soil from Thrislington Common and on top peat.

Hours after experiment started	Previously killed seeds on Thrislington Common top soil			Previously killed seeds on peat top soil		
	Uptake in Counts per minute	% accuracy	S	Uptake in Counts per minute	% accuracy	S
.5	213.0	7.0	.550	73.8	15.0	.524
1.0	363.8	5.0	.649	74.6	15.0	.681
1.5	1095.0	3.0	.701	74.2	15.0	.707
2.0	315.6	7.0	.677	60.8	15.0	.640
2.5	716.4	5.0	.690	88.0	10.0	.549
3.0	239.6	7.0	.699	67.6	15.0	.680
3.5	855.0	3.0	.608	74.2	15.0	.597
4.0	434.4	5.0	.631	81.6	10.0	.670
6.0	1248.2	3.0	.686	77.4	15.0	.666
8.0	780.2	5.0	.647	96.0	10.0	.642
10.0	2296.6	2.0	.675	83.2	10.0	.635
12.0	526.8	5.0	.674	334.2	5.0	.662
16.0	1737.0	3.0	.636	99.4	10.0	.672
22.0	2539.8	2.0	.694	125.4	10.0	.645
28.0	4094.2	1.5	.681	158.8	10.0	.638
36.0	1800.2	3.0	.678	125.8	10.0	.633
46.0	2819.2	2.0	.605	234.0	7.0	.643
58.0	1950.2	3.0	.733	164.0	7.0	.650
70.0	2555.6	2.0	.662	477.8	5.0	.651

TABLE 50:

Radio-chemical comparison of soils before and after shaking and centrifuging with distilled water.

Sample	Soil Type	Treatment	Wt. of soil in g.	Counts/ min.	Counts/ min./ g.	% accuracy of count	S (of Count)
A	Thrislington	Shaken & centrifuged with 10cm^3 water	2.57	952.4	370.58	3.0	.000
B	Peat	Shaken & centrifuged with 10cm^3 water	1.64	6370.4	3884.3	1.5	.000
C	Thrislington	Shaken & centrifuged with 10cm^3 water	2.00	67263.8	33631.9	0.5	.086
D	Peat	Shaken & centrifuged with 10cm^3 water	1.90	9062.0	4769.5	1.0	.002
E	Thrislington	Shaken & centrifuged with 20cm^3 water	2.66	47620.0	17902.0	0.5	.038
F	Thrislington	Shaken & centrifuged with 20cm^3 water	2.52	67482.4	26778.7	0.5	.081
G	Thrislington	Control	1.98	315262.2	159223.3	0.2	.067
H	Peat	Control	1.06	23331.2	22010.6	0.7	.000

Results from wash after centrifuging

	Counts/min.	Counts/min./ g. soil	% accuracy of count	S (of count)
Wash from soil sample A	51836.8	20107.6	0.5	0.589
Wash from soil sample B	28988.8	17646.8	0.5	0.568
Wash from soil sample C	45166.2	22353.6	0.5	0.652
Wash from soil sample D	26487.6	13809.4	0.7	0.641
Wash from soil sample E	37154.6	13894.6	0.5	0.710
Wash from soil sample F	29745.8	11769.8	0.7	0.672

TABLE 51:

Radio-chemical comparison of seeds before and after shaking with distilled water.

Treatment	Counts/min	% accuracy	S
Control	3391.4	2.0	.559
Shaken for 10 min. with $10cm^3$ distilled water	2893.8	3.0	.505
Shaken for 30 min. with $10cm^3$ distilled water	1566.6	3.0	.598

Computer programmes used for analyzing results.

The basic programmes are included here. It was sometimes necessary to alter them slightly ; for instance, to allow for different controls in calculating shoot indices or to alter axes is graphs. The alterations are not included.

1. Programme to calculate means and standard deviations of shoot lengths, shoot indices, and means and standard deviations of shoot indices.

```
PAGESIZE          NOEJECT
RUN NAME          B11W ANALYSIS
VARIABLE LIST     TCB.TCM.TCT.TB.TM.TT.WB.WM.WT.LB.LM.LT1.PB.PM.PT
INPUT FORMAT      FIXED(1X.F3.1.1x.F3.1.1x.F3.1.2x.F3.1.1x.F3.1.1x.
                  F3.1.2X.F3.1.1X.F3.1.1X.F3.1.2X.F3.1.1X.F3.1.
                  1X.F3.1.2X.F3.1.1X.F3.1.1X.F3.1)
INPUT MEDIUM      DISK
N OF CASES        15
COMPUTE           RTCB=TCB/1.63
IF                (RTCB GT 1.0) RTCB=1.0
COMPUTE           RTCM=TCM/.82
IF                (RTCM GT 1.0) RTCM=1.0
COMPUTE           RTCT=TCT/.57
IF                (RTCT GT 1.) RTCT=1.0
COMPUTE           RTB=TB/1.4
IF                (RTB GT 1.0) RTB=1.0
COMPUTE           RTM=TM/1.57
IF                (RTM GT 1.0) RTM=1.0
COMPUTE           RTT=TT/1.2
IF                (RTT GT 1.0) RTT=1.0
COMPUTE           RWB=WB/1.85
IF                (RWB GT 1.0) RWB=1.0
COMPUTE           RWM=WM/1.54
IF                (RWM GT 1.0) RWM=1.0
COMPUTE           RWT=WT/1.42
IF                (RWT GT 1.0) RWT=1.0
COMPUTE           RLB=LB/1.40
IF                (RLB GT 1.0) RLB=1.0
COMPUTE           RLM=LM/.90
IF                (RLM GT 1.0) RLM=1.0
COMPUTE           RLT1=LT1/1.40
IF                (RLT1 GT 1.0) RLT1=1.0
COMPUTE           RPB=PB/1.10
IF                (RPB GT 1.0) RPB=1.0
COMPUTE           RPM=PM/.60
IF                (RPM GT 1.0) RPM=1.0
COMPUTE           RPT=PT/.44
IF                (RPT GT 1.0) RPT=1.0
ASSIGN MISSING    RTCB.RTCM.RTCT.RTB.RTM.RTT.RWB.
                  RWM.RWT.RLB.RLM.RLT1.RPB.RPM.RPT. (99.9)
MISSING VALUES    TCB.TCM.TCT.TB.TM.TT.WB.WM.WT.LB.LM.LT1.PB.PM.PT  (99.9)
PRINT FORMATS     TCB.TCM.TCT.TB.TM.TT.WB.WM.WT.LB.LM.LT1.PB.PM.PT(2)
PRINT FORMATS     RTCB.RTCM.RTCT.RTB.RTM.RTT.RWB.RWM.RWT.RLB.RLM.
                  RLT1.RPB.RPM.RPT(2)
LIST CASES        CASES=1/VARIABLES=ALL
FREQUENCIES       GENERAL=TCB.TCM.TCT.TB.TM.TT.WB.WM.WT.LB.LM.LT1.PB.PM.PT
STATISTICS        1.5
READ INPUT DATA
FREQUENCIES       GENERAL=RTCB.RTCM.RTCT.RTB.RTM.RTT.RWB.
                  RWM.RWT.RLB.RLM.RLT1.RPB.RPM.RPT
STATISTICS        1.5
FINISH
```

Programmes

2. Programme to convert means of shoot indices to log. of 2,4-D concentration.

```
      DIMENSION A(10,15),B(8,15)
      N=15
      M=10
      MM=M-2
      READ(5,17)((A(1,J),1=1,M),J=1,N)
   17 FORMAT(8F6,3,2=9.1)
      DO 37 J=1,N
      DO 27 1=1,MM
      B(1,J)=(A(1,J)-A(M-1,J))/A(M,J)
   27 CONTINUE
   37 CONTINUE
      WRITE(6,47)
   47 FORMAT('   WK0   WK1   WK2   WK3   WK4   WK5   WK6   WK7  ')
      DO 67  J=1,N
       WRITE (6,57)(B(1,J),1=1,MM
   57 FORMAT(8F6.2)
   67 CONTINUE
      STOP
      END
```

3. Programme to plot graphs of radio-chemical experiments and (with alterations) weekly bioassays.

```
      DIMENSION X(100),Y(100),T1TLE(20)
      CALL PSPACE (0.1,  0.9,  0.1),   0.9)
      CALL MAP    (0.0,  80.0, 0.0.    4000.0)
      CALL AXES
      CALL BORDER
      CALL CTRSET(4)
      READ (5,100) TITLE
      READ (5,101) NL
      DO 30 IL=1,NL
      READ (5,101) NP
      DO 10 1P=1,NP
      READ (5,102) X(1P),Y(1P)
   10 CONTINUE
      CALL PTPLOT  (X,Y,1,NP,-2)
      CALL PTPLOT  (X,Y,1,NP,49+1L)
   30 CONTINUE
      CALL PSPACE  (0.0.1.0.0.0.1.0)
      CALL MAP     (0.0.1.0.0.0.1.0)
      CALL CTRSET(1)
      CALL PLOTCS  (0.1.0.92,TITLE,80)
      CALL GREND
      STOP
  100 FORMAT (20A4)
  101 FORMAT (13)
  102 FORMAT (2F10.0)
      END
```

Appendix 5 (ctd.)

4. Programme to calculate shoot indices from controls, to plot control graphs of shoot indices against log. of 2.4-D concentration.

```
      DIMENSION R(15.4)
      DIMENSION STYPW(15.4)
C      I:I-3=TC,4-6=T,7-9=W,10-12=L,13-15=P
C      J:1=100PPM,2=50PPM,3=10PPM,4=CONTROL
      DO 27 J=1,4
      READ(5,37)(STYPW(I,J),1,15)
 27   CONTINUE
 37  FORMAT (15F5.2)
      DO 57 J=1,4
      DO 47 I=1,15
      EVALUATE SHOOT INDICES
      R(1,J)=STYPW(1,J/STYPW(1.4)
      IF (R(1,J) .GT. 1.0)R(1,J)=1.0
 47   CONTINUE
      WRITE (6.67)
 67   FORMAT ('SHOOT  INDICES',/,'  TCB  TCM  TCT  TB  TM  TT  WB  WM
     1      WT   LB   LM   LT   PB   PM   PT')
      WRITE (6,77)((R(1,J),1=1,15),J=1,4)
 77   FORMAT (15F 5.2)
      STOP
      END
```

APPENDIX 5: computer programmes used to analyze results.

1. Programme to calculate shoot indices, and mean and standard deviations of shoot longths and shoot indices.

2. Programme to convert means of shoot indices to log. of 2,4-D concentrations.

3. Programme to plot graphs.

4. Programme to calculate shoot indices from controls.

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